THE UNIVERSITY OF QUEENSLAND

AUSTRALIA

A Critical Evaluation of the Effects of Plant Essential Oil Formulations against Silverleaf Whitefly, *Bemisia tabaci*, B Biotype (Gennadius) and its Natural Enemies

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A thesis submitted for the degree of Doctor of Philosophy at

The University of Queensland in 2016

School of Agriculture and Food Sciences

Abstract

The toxicity of 30 botanical products and mixtures were evaluated using preliminary bioassay tests to find out their overall effectiveness against the developmental stages of silverleaf whitefly (SLW) *Bemisia tabaci*, B biotype, (Gennadius) (Hemiptera: Aleyrodidae). Effective formulations were prepared to be tested in replicated experiments against the egg, nymphal and adult stages of SLW. From the results, mustard oil showed an ovicidal effect, lauryl glucoside, a surfactant, had produced high mortalities against the nymphal stages whereas monoethanolamine, diethylene glycol monomethyl ether (cellosolve acetate) and laureth – 7ethylene oxide-carboxylate (laureth carboxylate) had produced high to moderate level of adulticidal effects. Three formulations were prepared from those effective products and then used in replicated experiments testing their lethal (toxicity) and sublethal (repellency and oviposition deterrent) effects against SLW. Additionally, trials were conducted to investigate their impacts on one of the main SLW parasitoids, *Eretmocerus hayati* (Zolnerowich and Rose) (Hymenoptera: Aphelinidae).

Leaf dipping and spraying methods were used to assess the toxicity effects of the products, and choice and no choice repellence tests were used to determine the repellence index (RI) and oviposition deterrent index (ODI). A glass - slide bioassay was used to determine the lethal effects of the formulations against the SLW parasitoid. Different ranges of the tested rates of the formulations were prepared starting from 0.001% v/v to 10% v/v to investigate the proper effective rates that provide sufficient mortality rates of the developmental stages of SLW.

After promising results of the mixture containing mustard oil and liquid soap, replicated experiments were conducted against each developmental stage of SLW. When tested on eggs, at a concentration of 0.25%, mortality was 95.8%, whereas the mortality percentages were 43% and 50% at tested rates of the mixture at 0.1% and 0.05%, respectively. Mustard oil evaluated against nymphal stages was effective at a rate of 0.25% and above against young and old nymphs (86.4% and 47.4% mortality, respectively). Tests against the adult stage at 0.25% and 0.5% resulted in low mortality; 34.0% and 37.0%, respectively. However, at 1% and above mortality was high (94.17% at 1%).

On the basis of these results, three formulations were prepared: formulation one (F1) containing mustard oil, MW-100 emulsifier, lauryl glucoside and cellosolve acetate, formulation three (F3) containing mustard oil, MW-100 emulsifier, laureth carboxylate and monoethanolamine and formulation four (F4) containing mustard oil, MW-100 emulsifier, lauryl glucoside and monoethanolamine. These formulations were evaluated at different concentrations (0.25%, 0.44%.

0.69%, 1% and 1.23%). The formulations had an effective impact on the eggs of SLW, disrupting the embryogenesis process of the eggs. These formulations also affected all nymphal instars. However, the tested rates did not show sufficient effects on adult mortality.

When the formulations were evaluated for their repellent and oviposition deterrent effects, numbers of adults on the leaves were counted 2, 6, 12, 24 and 48 h after the adult introduction. From the results of choice tests, F1 had the highest RI value (0.18), whereas the RI value of F4 (-0.01) was the lowest among the tested formulations. F4 showed certain repellence and oviposition deterrence effects (RI= -0.01; ODI= -44). In no-choice experiments, the mean number of adults attracted to the lower side of the leaflets treated with the formulations F1, F3 and F4 was calculated. The results indicated that F3 and F4 showed a reduction in adult mean number of 34.1% and 46.9%, respectively, and accordingly there were a reduction in the mean number of laid eggs by 77.3% and 81.2%, respectively, compared with the control.

There were different responses of the formulations on the parasitoids. F1 had the lowest significant adverse effect on the parasitoid among the tested formulations: 11.11%. There were no significant differences between the tested rates on the parasitoids. F3 and F4 showed severe effects on the parasitoids. The parasitized mortality rates reached 60%.

From the above, the mixture of mustard oil and liquid soap could be used effectively against all developmental stages of SLW. The formulations also showed high mortality rates against egg and nymphal stages but no significant effects against adults. F1 had the highest RI among other formulations in choice tests however there were no significant differences between them in their effectiveness when tested in no choice repellence tests. F1 was safer to the SLW parasitoids than F3 and F4. Therefore, F1 containing mustard oil, MW-100 emulsifier, lauryl glucoside and cellosolve acetate could be used in future against SLW developmental stages and could be incorporated in integrated pest management programs (IPM).

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Publications during candidature

Conference abstract (oral presentation)

Obaidoon, Y, Senior L, & Hassan, E 2014, 'A preliminary study on the toxicity and disruption effects of essential oil formulations on the developmental stages of *Bemisia tabaci* B biotype', In *Proceedings of the International Conference on Biopesticides 7* (ICOB 7). 19 – 25 October 2014. Antalya, Turkey, P. 83.

Conference abstract (poster presentation)

Obaidoon, Y, Senior L, & Hassan, E 2015, 'Evaluation of the Toxicity and Developmental Effects of New Plant Essential Oil Formulations against the Eggs of *Bemisia tabaci* B Biotype', In *Proceedings of the XVIII International Plant Protection Congress,* 24 – 27 August 2015. Berlin, Germany.

Publications included in this thesis

No publications included.

Contributions by others to the thesis

Dr. Errol Hassan and Dr. Lara Senior supervised this research and critically reviewed the thesis. Mr Allan Lisle provided assistance with experimental designs and statistical analysis.

Statement of parts of the thesis submitted to qualify for the award of another degree

None.

Acknowledgements

First of all, I would like to express my deep gratitude to Adjunct Associate Professor Dr. Errol Hassan and Dr. Lara Senior, my principle and associate advisors, for their patient, guidance, enthusiastic encouragement and constructive suggestions during my study at The University of Queensland, Gatton Campus, Australia. Also my sincere thanks go to Associate Professor Victor Galea and Senior Lecturer Dr. Doug George for their valuable advice on this research project during the milestone reviews. I also wish to acknowledge Allan Lisle for his guidance in statistical analyses. I would like to offer my special thanks to Richard Lloyd, from Toowoomba Research Centre, for his assistance in providing and establishing the silverleaf whitefly, *Bemisia tabaci*, B biotype colony. My appreciation goes to Mel Schneemilch, Lachlan Fowler, Heidi Robertson and Ms. Bridgett for their technical assistance. My grateful thanks are extended to Allan Twomey from BioAust Pty Ltd for supplying plant essential oils and formulations. A great appreciation goes to my senior colleague, Prof Dr. Nabil Abdel Salam, Plant Protection Expert in Royal Court Affairs in Oman, for his helpful advices. Special thanks to the Omani Government for awarding me a PhD scholarship. My greatest thanks convey to my parents, brothers and sisters for their constant support and encouragement. A grateful appreciation goes to my lovely wife, Nuha, and my four children, Amrou, Al-Hanoof, Omaer and Al-Hatoon, for their love, patience, understanding and encouragement. Finally, I would like to convey my thankfulness to my kind friends, Salem Al-Alawi, Rabie Al-Bosaidi, Awadh Al-Haj, Abdelqader Ibrahim, Emad Saar, Badar Al-Barhi, Saleh Al-Jahdhami and Yaqoob Al-Hosni for their supportive assistances.

Keywords

Silverleaf whitefly, *Bemisia tabaci,* essential oil formulation, mortality rate, repellent, oviposition deterrent, *Eretmocerus hayati*,

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 070603 (Horticultural Crop Protection) – 100%

Fields of Research (FoR) Classification

FoR code: 0706 (Horticultural Production) – 100%

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List of abbreviations used in the thesis

AAI	After Adult Introduction
ANOVA	Analysis of Variance
EAN	Estimated Adult Number
LD	Lethal Dose
ODI	Oviposition Deterrent Index
RI	Repellence Index
SLW	Silverleaf Whitefly

CHAPTER 1: General Introduction

1.1. Background

The silverleaf whitefly (B biotype), *Bemisia tabaci* (Gennadius), (Hemiptera: Aleyrodidae) is one of the most serious agricultural insect pests, affecting crop plants as hosts such as tomatoes, cotton, cassava and beans, as well as ornamentals. Of importance is the fact that they have worldwide distribution and as such are commonly known insect pests and vectors to entomologists, virologists, agriculturists and growers (Oliveira et al. 2001; De Barro et al. 2011; Thompson, 2011). It was described in 1889 as a tobacco whitefly (*Aleyrodes tabaci*). A detailed description of *B. tabaci* has been provided by Bellows et al. (1994). Drost et al. (1998) and Oliveira et al. (2001) reviewed that *B. tabaci* biotypes have been recorded from more than 600 different plant species. The host plants include field crops, ornamentals, vegetables and fruit crops. Additionally, some weeds serve as alternative hosts.

The whitefly causes damage to the plant directly by sucking the plant sap and indirectly by transmitting viruses. Duffus (1987) and Jones (2003) have stated that several virus groups can be transmitted by whitefly including: geminiviruses, closteroviruses, carlaviruses, potyviruses, nepoviruses, luteoviruses and DNA-containing rod-shaped virus. Another way in which whitefly causes damage is by excreting honeydew on the leaves and fruits which is associated with sooty mould production (Byrne and Bellows 1991). This can cause a reduction in the quality and quantity of the crop.

Biological control plays an important role in suppressing whitefly populations (Naranjo and Ellsworth 2009a). Parasitoids have been frequently studied. For example, *Encarsia* sp. and *Eretmocerus* sp. (Hymenoptera: Aphelinidae) are the main hymenopteran parasitoids that are used against whitefly (López and Andorno 2009; Yang and Wan 2011; Zang and Liu 2008; De Barro and Coombs 2009; Villanueva-Jimenez et al. 2012). Studies have been conducted using predators such as *Amblyseius swirskii* Athias-Henriot (Mesostigmata: Phytoseiidae) (Nomikou et al. 2002; Xiao et al. 2012) and shown some promising results. Nonetheless, efforts to control this important pest biologically have not ceased and numerous studies on the enemy fauna as well as on the technology necessary for achieving biological control are continuing.

On the other hand protecting crops from smaller insect pests, such as, whitefly species with synthetic insecticides is difficult. There are some challenges involved in preventing and controlling infestation of whiteflies on host plants. One of these is caused by the fact that whiteflies stay on the

underside of leaves, making them less accessible for insecticide foliar sprays. But more importantly, they have developed a high resistance to many synthetic insecticides such as organophosphates and pyrethroids, used in agriculture (Denholm et al. 1998; Ma et al. 2007; Erdogan et al. 2008; llias et al. 2012). In addition, using insecticides to control this pest species comes with some serious additional difficulties. Growing concerns about the high risks involved with using conventional toxic insecticides are causing an increased interest to find environment-friendly alternatives to control these pests (Al Lawati et al. 2002; Kulkarni et al. 2009).

Essential oils are volatile liquids, or semi-liquids, typically forms with the complex mixtures of volatile compounds produced as secondary metabolites in plants (Nakatsu et al. 2000; Regnault-Roger et al. 2012; Saad et al. 2013). They have recently gained interest as potential source for the development of "Bioipesticides", and as environmentally friendly alternatives to conventional synthetic insecticides. They possess bioactive compounds and can be used to control different type of insect pests as repellents, fumigants, anti-feedants, ovipositing deterrents, chemosterilants, and or toxins (Regnault-Roger 1997; Isman 2000; Isman 2006; Sertkaya et al. 2010; Regnault-Roger et al. 2012). Several essential oil-based products derived from the different plant species such as, *Azadirachta indica* A. Jass. (Meliaceae), (Pinheiro et al. 2009), *Thymus vulgaris* L. (Lamiacae) and *Pogostemon cablin* Blanco (Lamiaceae) (Yang et al. 2010), and *Allium sativum* L., (Amaryllidaceae) (Liu et al. 2014) have been screened against the silverleaf whitefly. The results of these studies showed that the plant essential oils can be useful as potential control agents against the *B. tabaci*. Further a most recent study demonstrated that the essential oils of *Piper callosum* Ruiz and Pav. (Piperaceae), *Adenocalymma alliaceum* Lam. (Bignoniaceae), *Pelargonium graveolens* L'Her. (Geraniaceae), and *Plectranthus neochilus* Schltr. (Lamiaceae) inhibit the settlement and oviposition of *B. tabaci* biotype B adults in tomato plants. In fumigation tests, *A. alliaceum* essential oils showed effects against the nymphs and adults of *B. tabaci* biotype B, respectively (Fanela et al. 2016). Alternatively, surfactants which are important additives in numerous agrochemicals and biological formulations including health care products (McDonnell and Russell 1999) may also be used in suppressing soft bodied agricultural pests including whiteflies (Liu and Stansly 1995; McKenzie et al. 2005).

Integrated pest management (IPM) uses a number of complementary control methods to suppress pest populations (Castle and Naranjo 2009). IPM programs to control whitefly, *Bemisia tabaci* have been studied (Ellsworth and Martinez-Carrillo 2001; Naranjo and Ellsworth 2009b) and shown an obvious reduction in whitefly population.

1.2. Insecticide Resistance

Insecticide resistance is defined by Denholm and Devine (2013) as an evolutionary adaptation conferred by genes encoding modified receptor enzymes that detoxify insecticides. In the USA, pesticide resistance costs about $ 1.5 billion per year. The main reason for resistance is the highly frequent use of insecticides from the same class and their application as a primary option to control pests (Pimentel 2005). Another reason is that the high reproductive rate of *B. tabaci* leads to increased mutation rates within populations (Castle et al. 2010).

Because of the frequent use of insecticides, *B. tabaci* has developed resistance to most conventional insecticides (Palumbo et al. 2001). In spite of this disadvantage, the effectiveness of chemical control can be obtained by using it as a part of IPM programs (Gerling and Naranjo 1998; Ellsworth and Martinez-Carrillo 2001; Stansly et al. 2004; Naveed et al. 2008). Resistance to conventional insecticides including organophosphates, carbamates and pyrethroids has been recorded. In addition to that, it also showed resistance to novel insecticides such as neonicotinoids and insect growth regulators (Byrne and Bellows 1991; Perring 2001; Erdogan et al. 2008). To manage insecticide resistance, different insecticide groups with different modes of actions are applied in rotation. In general, the efficient use of other control methods including cultural and biological control methods beside the chemical methods decreases the chances of insecticide resistance (Palumbo et al. 2001).

1.3. Synergism

Synergistic action can be achieved by using a combination of different components to increase the effectiveness of the resultant material. In this study, different formulations of essential oils and surfactants were used against silverleaf whitefly, B biotype developmental stages. Because of the effectiveness of essential oils as repellents, anti-feedants, ovipositing deterrents or lethal agents (Farghaly et al. 2009; Pinheiro et al. 2009), they can be used as alternatives to conventional insecticides. In addition, the surfactants are mainly used as emulsifiers, solubilizers, wetting and cleaning agents (McDonnell and Russell 1999). Thus, the use of the combination of both essential oils and surfactants is to achieve a synergistic effect.

Mixtures of essential oils and surfactants could play an important role in programs for control of whiteflies (Liu and Stansly 1995; 2000; Liu et al. 1996). The surfactants that belong to sugar esters such as sucrose octanoate, showed a potential for reducing whitefly infestations (McKenzie et al.

2005). The surfactants, which are used in these formulations, include 50% decyl glucoside, 50% capryl glucoside, 50% lauryl glucoside and a mixture of 50% lauryl sucroside and 50% glycoside. Beforehand, to establish efficacy, the different rates of the surfactants were tested against all developmental stages of silverleaf whitefly as non-replicated experiments.

1.4. Objectives of the study

The main objective of this research was to evaluate the effectiveness of essential oil formulations against silverleaf whitefly, *B. tabaci* B biotype. More specific goals were:

- To investigate the insecticidal properties of essential oils from plant origin;
- To determine acute toxicity and sublethal developmental effects of some essential oils on eggs, nymphal and adult stages;
- To investigate repellence effects of some essential oils using leaf choice and no-choice bioassays;
- To identify and determine the mode of action of plant extracts on oviposition of silverleaf whitefly;
- To identify and determine the mode of action of plant extracts on development and survival of silverleaf whitefly from different nymphal stages to adult emergence; and
- To investigate the effectiveness of biopesticides under glasshouse conditions.

CHAPTER 2: Review of Literature

2.1. Introduction

This review describes aspects of the biology, pest status and management of the silverleaf whitefly, *Bemisia tabaci* B biotype. Information on biological parameters including classification, description and life cycle of the silverleaf whitefly is cited in detail in order to assist in designing the methodology in this study. In addition, information on the importance of *B. tabaci* B biotype as a pest and how it causes damage to plant hosts is reviewed. Furthermore, control methods used against the silverleaf whitefly are mentioned in this review including cultural, biological and chemical control methods. Due to the adverse effect of the conventional insecticides, biopesticides, such as botanical essential oils, are used as an alternative option and incorporated in IPM programs. The main objective of this review was to establish and evaluate the present literature available on silverleaf whitefly to develop the parameters of my research.

2.2. Biology of *Bemisia tabaci* B biotype

2.2.1. Classification of *Bemisia tabaci* B biotype

The silverleaf whitefly, *Bemisia tabaci* (Gennadius) belongs to subfamily: Aleyrodinae, family: Aleyrodidae, superfamily: Aleyrodoidea, suborder: Sternorrhyncha and order: Hemiptera. It has piercing-sucking mouthparts and feeds on the phloem sap (Brown et al. 1995; De Barro et al. 2011) as do other homopteran insects such as aphid, mealybug and scale insects.

From previous literature reviews, it is clear that the classification of whitefly *B. tabaci* (Gennadius) underwent two main historical periods, the first one being the *B. tabaci* nomenclature itself which took place between 1889 and 1933. The second period was its biotype identification (1950s – until the present) (Brown et al. 1995; De Barro et al. 2011).

In 1889, *B. tabaci* was first found in Greece in tobacco plants. It was identified as *Aleyrodes tabaci* (Gennadius, 1889) and named as the tobacco whitefly. Eight years later (1897), the whitefly was found in sweetpotato plants in the USA. It was called *Aleyrodes inconspicua* Quaintance and named as the sweetpotato whitefly (Quaintance, 1900). A third important time in whitefly naming was in Brazil in 1928, when whitefly found on *Euphorbia hirtella* Boiss (Euphorbiaceae) plants and

described as *Bemisia costalimai* Bondar (Mound and Halsey 1978). Additionally, in Taiwan in 1933, whitefly was collected and named as *Bemisia hibisci* Takahashi (Mound and Halsey 1978; Oliveira et al. 2001). It is also commonly named as cotton and poinsettia whitefly.

The second era of *B. tabaci* nomenclature started in the 1950s. It was not possible to distinguish between different *B. tabaci* populations using morphological techniques (Brown et al. 1995; Rosell et al. 1997; Perring, 2001; Oliveira et al. 2001; De Barro et al. 2011). Bellows et al. (1994) described two *Bemisia* species, *B. tabaci* (Gennadius) and *B. argentifolii* (Bellows and Perring) using the morphology of the fourth instar nymph (pupal case). *B. argentifolii* was identified by the absence of a dorsal seta, the width of the thoracic tracheal folds and the width of the wax extrusions from the tracheal folds. However, this method is not now used due to the appearance of different morphological characters in the pupal case of the same species when attacking different host plants (Gerling and Mayer 1996; Gill and Brown 2010).

In the past 20 years, molecular markers have been used to define *B. tabaci* biotypes (Liu et al. 2012). The main genetic techniques were allozymes (Gunning et al. 1997), RAPD PCR (randomly amplified polymorphic DNA polymerase chain reaction) (De Barro and Driver 1997), AFLP (amplified fragment length polymorphism) (Cervera et al. 2000), rITS1 (ribosomal integenic transcribed spacer 1) (De Barro et al. 2000) and mitochondrial DNA markers, mt16s and mtCo1) (Frohlich et al. 1999; Ma et al. 2009). From the above studies of the phylogenetic characteristics of *B. tabaci* biotypes, De Barro et al. (2011) have listed 36 identified biotypes worldwide. These are A, AN, B, B2, BR, C, Cassava, Cv, D, E, F, G (India), G (Guatemala), H, I, J, *Jatropha*, K, L,M, N, NA, Okra, P, PCG-1, PCG-2, PK1, Q,R, S, Sida, SY, T, ZHJ1, ZHJ2, and ZHJ3. Furthermore, those studies indicate that *B. tabaci* is a species complex (Liu et al. 2012). Gill and Brown (2010) suggested that from the genetic researches, the origins of many biotypes have been provided. Therefore, the question about whether *B. tabaci* is a complex of different species or different biotypes is not yet answered (Brown et al. 1995; De Barro et al. 2011).

2.2.2. Description of *B. tabaci* B biotype

The whitefly *Bemisia tabaci* was described morphologically in detail by Bellows et al. in 1994. *B. tabaci* is a tiny insect. It undergoes incomplete metamorphosis. Its life is divided into six stages: egg, 1st instar (crawler), 2nd instar, 3rd instar, 4th instar nymphs (pupa) and adult (Byrne and Bellows 1991). Females lay oval eggs on the lower surface of the leaf attached by a pedicel. The pedicel is usually inserted in the stomatal opening and provides attachment and a supply of moisture to the

egg (Byrne and Bellows 1991; Buckner et al. 2002). Eggs could be laid singly or in small groups (Kumarasinghe et al. 2009). The egg starts pale yellow, becoming brown in color when it is close to hatching. At about 26℃, *B. tabaci* B biotype's female laid approximately 200 eggs during its life cycle at an average of six eggs per day (Martinez et al. 2009). The number of eggs laid may vary depending on temperature and host plant. Butler et al. (1983) observed that females did not lay eggs at 14.9℃.

The 1st instar nymph is also called a crawler. This is the only immature stage which is mobile. It moves less than 30 mm from the egg shell before it settles down and penetrates its stylet into the leaf tissues and feed on the phloem sap. The stylet consists of two canals. One canal is to supply saliva and the other to suck fluid (Gullan and Cranston 2000). The 2nd and 3rd instar nymphs are yellow in color and oval or elongate–oval in shape (Byrne and Bellows 1991). The 4th instar (pupa), is colorless with a thin layer of wax. It has two red eye spots and they can be seen clearly through the transparent cuticle. (Bellows et al. 1994; Islam and Ren 2007).

The newly emerged adult (female = 0.85 mm in length and male = 0.80 mm in length) has a yellow abdomen and hyaline wings (Bellows et al. 1994). During the first 10 hours from emergence, the adult covers itself with white wax produced from the ventral gland in the abdomen. The adult is then ready for feeding and mating (Li et al. 1989). A mated whitefly produces male and female eggs, whereas unmated whiteflies produce only male eggs (Gullan and Cranston 2000).

Recently, in China, Li et al. (2013) described the morphology and morphometry of *B. tabaci* B biotype using microscopes, where insects had been reared on cotton plants under laboratory conditions at 27±1 °C, 14 L:10 D photoperiod and 70±10% relative humidity (n=20). Table 2.1 includes measurements of an average body size (μm) and the mean duration in days of each developmental stage. Under the same laboratory conditions, silverleaf whitefly reared in The University of Queensland (UQ), Gatton Campus, body sizes of *B. tabaci*, B biotype were measured and given in Table 2.1 and compared with Chinese measurements.

2.2.3. Life cycle and host range of *B. tabaci* B biotype

The life cycle of *B. tabaci* B biotype depends on temperature and host plant (Sharaf et al. 1985; Drost et al. 1998; Bosco and Caciagli 1998; Chaudhuri et al. 2001; Islam and Ren 2007; Kumarasinghe et al. 2009).

The following table (2.2) details the developmental time of *Bemisia tabaci* when cultured on different hosts at different temperatures.

Table 2.1: Length and width (µm) measurements of body size and the mean duration of each developmental stage of *B. tabaci* B biotype (Li et al. 2013).

	China		Australia (UQ, Gatton)		(China an Australia)
Developmental stage	Length (µm)	Width (µm)	Length (µm)	Width (µm)	Mean duration (Days)
Egg	250	150	200	100	7
1st instar nymph	269	154	250	150	8
2nd instar nymph	390	240	400	250	5
3rd instar nymph	802	541	800	550	4
4th instar nymph (Pupae)	865	600	850	600	6
Adult female	855		850		18
Adult male	779		800		13

Table 2.2: SLW developmental time in days at different host plants from egg to adult stage.

Host Plant	**T (°C)**	**Developmental Time (Days)**	**Reference**
Cotton	Summer Winter	17 74.5	Azab et al. 1971
Bean	26±2 26 16	34 22 70	Martinez et al. 2009 Bosco and Caciagli 1998
Tomato	23 28	40 19.5	Chaudhuri et al. 2001
Cassava	29±2	37.5	Kumarasinghe et al. 2009
Eggplant	30 15	14 105	Wang andTsai 1996

Furthermore, Han et al. (2013) studied the developmental time of the sweet potato whitefly, *Bemisia tabaci* Q biotype. They found, for example, in eggplant that egg developmental time was 27 days at 15℃ and 5 days at 30℃. Developmental time of the nymphal stage was also measured and it took 73 days at 15℃ whereas it spent 12 days at 27.5℃. Kumarasinghe et al. (2009) have

identified the duration of each developmental stage of *B. tabaci* B biotype in cassava plants under laboratory conditions at 29±2 °C (Figure 2.1). The mean duration of different stages is given in figu

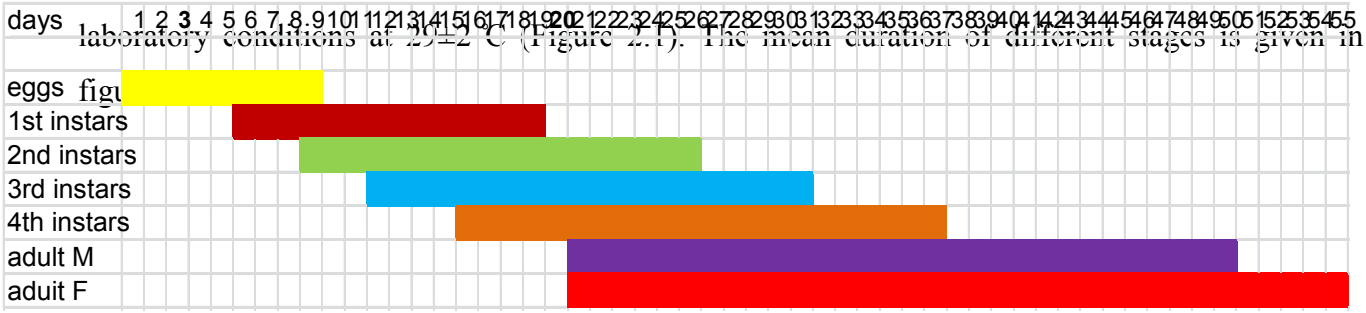


Figure 2.1: The duration of each developmental stage of *B. tabaci* B biotype in cassava plants (Kumarasinghe et al. 2009).

The silverleaf whitefly (SLW), *Bemisia tabaci* B biotype is polyphagous. It has a wide range of host plants including field crops, fruit, vegetables, ornamentals and weeds. Oliveira et al. (2001) have reviewed that *B. tabaci* has been recorded from more than 600 different plant species. In Pakistan, 160 plant species belonging to 42 families were recorded (Attique et al. 2003). Host preference of *B. tabaci* has been tested in several studies. Preferences might be due to the morphological features of the host leaf (Bezerra et al. 2004). *B. tabaci* prefer eggplant more than okra, tomato and chilli as indicated by the mean numbers of eggs, nymphs and adults (Mansour et al. 2012). Khan et al. (2011) have also evaluated host plant selection of *B. tabaci* in three host plants: eggplant, tomato and chilli. The *B. tabaci* selected eggplant. It fed and laid more eggs than in the other two host plants.

Eggplant leaves are more hairy with thick trichomes (Khan et al. 2011). Even within the varieties of a host plant, *B. tabaci* showed some preferences such as tomato varieties (Setiawati et al. 2009; Oriani et al. 2011) and eggplant varieties (Islam et al. 2010). Islam et al. (2010) have studied host preference by focusing on adult feeding, oviposition, and developmental time of *B. tabaci*. They found that eggplant variety "Baiyu" is less susceptible than the varieties, ‘Dafeng’ and ‘Beisite. Host susceptibility refers to the ability to then complete the life cycle in short time and lay eggs abundantly (van Lenteren and Noldus 1990). The silverleaf whitefly, in general, prefers to attack plants with hairy instead of smooth leaf surfaces (Islam et al. 2010).

Adults (male & female)
(0.8 mm & 0.85 mm)
(13 & 18 Days)

Eggs
(0.2 mm x 0.1 mm)
7 Days

4 th nymphal instar (Pupal stage)
(0.85 mm x 0.6 mm)
6 Days

1 st nymphal instar
0.25 mm x 0.2 mm)
8 Days

3 rd nymphal instar
(0.7 mm x 0.5 mm)
4 Days

2 nd nymphal instar
(0.4 mm x 0.25 mm)
5 Days

SLW Life Cycle
at 27 C and14L:10D

Figure 2.2: SLW life cycle with an average developmental time of each stage at 27°C and 14L: 10D - cultured at UQ, Gatton Insectary (Photos by Yasir Obaidoon).

2.3. Pest Status

The silverleaf whitefly, *B. tabaci* B biotype is a serious agricultural pest worldwide. It causes damage to the plant directly by sucking plant sap, by excreting honeydew which is associated with sooty mould production and by transmitting viruses (Duffus 1987; Byrne and Bellows 1991; Oliveira et al. 2001; Jones 2003). This causes a reduction in the quality and quantity of the crop.

In Pakistan, *B. tabaci* was first reported as a serious pest of cotton in the early 1930s (Misra and Lamba 1929). In the USA, *B. tabaci* B. biotype caused severe damage to ornamental plant species in the 1950s (Costa and Brown 1991). In Arizona, California and Texas between 1994 and 1998, about US$154 million was spent to control *B. tabaci* in cotton fields (Ellsworth et al. 1999). Cotton growers in the USA face losses of up to US$500 million annually. In Brazil, since 1995, most of the main crops, such as beans, tomatoes, cotton, melons, okra and cabbage have been severely infested by *B. tabaci*. The estimated loss was more than US$5 billion (Lima et al. 2000).

Whiteflies feed on phloem sap of the plants which causes a reduction in plant growth and yield (Byrne and Bellows 1991). The feeding can cause yellow mottling on the leaves which is a problem, for example, in ornamentals, and high populations can even lead to death of the plants. In some plants the toxins in the whitefly saliva can cause problems, e.g. uneven ripening in tomatoes that makes the crop unmarketable or reduces its value (Byrne and Miller 1990).

After feeding on the plant phloem sap, whiteflies excrete honeydew (Byrne and Bellows 1991). It is mainly composed of a disaccharide, trehalulose, which is not a part of the phloem sap (Byrne and Miller 1990). Honeydew serves as a medium for sooty mould fungi such as *Capnodium* spp., which turns leaves black in color and is sticky (Perkins 1983). The accumulation of honeydew affects the quality and quantity of crop production. Cosmetic damage alone can make fruit, vegetables and ornamentals unmarketable or cause issues as they have to be cleaned prior to marketing (Ellsworth 1999).

Besides its direct damage to crops, whitefly acts as a vector of many plant viral diseases (Duffus 1987; Oliveira et al. 2001; Jones 2003). *Bemisia tabaci* became an important vector of plant virus diseases since the early 1980s (De Barro 1995). *Bemisia tabaci* transmits 111 plant viruses (Jones 2003). The main virus groups which are transmitted by *B. tabaci* include: begomoviruses, closteroviruses, carlaviruses, potyviruses, nepoviruses, luteoviruses and DNA-containing rod-shaped virus (Duffus 1987; Morales 2006). Among them, 90% of the plant viruses belong to begomoviruses (Jones 2003). This group of viruses causes crop losses from 20% to 100% (Brown and Bird 1992).

Plant viral diseases that are transmitted by whitefly have caused damage to cassava, tobacco, tomatoes, okra, cotton, melon, watermelon, beans, soybeans, squash, peppers, lettuce, papaya (Oliveira et al. 2001) and sweet potato (Valverde et al. 2004). Tomatoes are severely affected by Tomato Yellow Leaf Curl Virus (TYLCV) worldwide (Oliveira et al. 2001). A 100% yield loss has occurred due to the TYLCV in tomato plantations. Upward curling of leaflet margins, reduction of leaflet area, yellowing of young leaves, stunting and flower abortion are the main symptoms that are related to TYLCV infection (Moriones and Navas-Castillo 2000).

In Africa, cassava is a major food source. It is affected severely by whitefly. The most serious viral disease transmitted by whitefly is Cassava Mosaic Disease (CMD). It is caused by a complex of cassava mosaic geminiviruses (Harrison et al. 1997). The yield losses can reach 95% in cassava fields (Legg 1999). In India, in the northern cotton production area, cotton was infected by Cotton Leaf Curl Virus (CLCV) which decreased production by 75% in 1998 compared to 1990 (Singh et al. 1999).

In Oman, the information about whitefly is very limited. This pest has been present since the 1990s causing many plant protection problems in the vegetable sector, especially in tomatoes. Conventional insecticides are used to control it but due to its resistance, it became difficult to control it effectively in Oman. Attempts were made to introduce parasitic wasps from the UK in greenhouses to control this pest. However, this has only been effective to a certain degree.

In Australia (Queensland), *B. tabaci* biotype B was first identified in 1959 (Gunning et al. 1995). It was reported in cotton fields in 1994 (Carver and Reid 1996). De Barro and Driver (1997) distinguished the B biotype from other biotypes using RAPD-PCR technique. In 2001, an outbreak of *B. tabaci* biotype B occurred in cotton fields grown in an area around the township of Emerald (23°23' S, 148°10' E) in central Queensland (Moore et al. 2004). After a year, field sampling and control strategy was implemented in the central Queensland cotton fields. The control program that was used included the insect growth regulator (IGR) pyriproxifen which was applied according to the action thresholds of 3-5 adults per leaf and 0.5-1 nymphs per 3.88 cm^2. Subsequently, high population densities of whitefly have infested horticultural crops and weeds (Ellsworth and Martinez-Carrillo 2001; Sequeira and Naranjo 2008).

Due to the excess use of insecticides, whitefly has become resistant to many of them including the conventional insecticides, neonicotinoids and pyriproxifen (Costa et al. 2003; Horowitz et al. 2005; Luo et al. 2010). An IPM program against whitefly has been conducted in north-eastern Australia grain fields (Brier et al. 2008). It has been suggested to concentrate on biological control methods especially the use of the parasitoid *Eretmocerus hayati* Zolnerowich and Rose (Brier et al. 2007).

From the above, it is clear that the silverleaf whitefly, *B. tabaci* is one of the most common and damaging agricultural pests worldwide. All kinds of insecticides have been used to manage it. However, it develops insecticide resistance rapidly. The main objective of this thesis is to investigate the potential insecticidal effectiveness of some formulations consisting of essential oils combined with surfactants on the developmental stages of *B. tabaci*. In addition to that, another objective is to test these formulations against its parasitoid.

2.4. Management of *Bemisia tabaci* B biotype

Whitefly *B. tabaci* is one of the most dangerous insect pests to agricultural crops. Furthermore, the use of chemical control, such as organophosphates and pyrethroids, as a primary option to deal with whitefly has resulted in the development of insecticide resistance (Denholm et al. 1998). In addition, there are risks of synthetic insecticide application to humans, the environment and natural enemies of the pests, and therefore alternative and novel methods to control this pest species will need to be researched.

Use of yellow sticky traps to monitor and, therefore, predict whitefly density can help in chemical control decision-making (Pinto-Zevallos and Vanninen 2013). Action threshold is defined as the levels of pest density or damage causing measurable losses in yield or quality (Schuster 2005). In the case of whitefly, it can be measured by the number of whitefly adults caught per trap per week. It varies from crop to crop (Ndomba 2007).

Integrated pest management tools used against agricultural pests include different control methods to suppress pest populations (Castle and Naranjo 2009; Ellsworth and Martinez-Carrillo 2001; Naranjo and Ellsworth 2009b). The main four control methods implemented in IPM programs are cultural, host plant resistance, biological and chemical control (Hilje et al. 2001).

2.4.1. Cultural control methods

Cultural practices can play an important role in integrated pest management programs against whitefly. Hilje et al. (2001) detailed the main cultural tactics that can be used in whitefly management including: crop free period, changing planting date, exclusion, intercropping, fertilizing, mulching and optimising irrigation.

A crop free period assists in whitefly population reduction. It should be synchronized with weed and crop residue disposal to reduce availability of alternative hosts. In Sudan in the Gezira region, cotton leaf curl disease was controlled by cotton plant free fields for two months and removing ratoon growth (Bailey 1930). Another example of the importance of the crop free period was in south central Africa: when tobacco was not planted and ratoon growth removed, tobacco leaf curl disease was controlled (Cock 1986).

In the Dominican Republic in the Azua valley and other tomato production areas, tomato production was 21.6 ton/ha in 1989. However, in 1992, tomato was severely attacked by tomato yellow leaf curl virus (TYLCV). The next year (1993), production dropped to 11.3 ton/ha. To solve this problem, planting of whitefly hosts was banned for three months before the tomato growing season (Alvarez and Abud-Antun 1995; Villar et al.1998). In 1997, four years after applying this tactic, the production was raised to 30.4 ton/ha.

Planting dates either early or late help to avoid whitefly and virus inoculum (Hilje et al. 2001). For example, Bi et al. (2005) observed that adult whitefly population level was higher on early planted cotton than on late planting. Mohamed (2012) tested different planting dates of cucumber. An early planting date resulted in a lower population of *B. tabaci* nymphs.

Crop exclusion can help in protecting the crop from whitefly incidence. Young seedlings are more susceptible to whitefly and virus damage. Covering the crop temporarily at an early stage with materials, such as mesh or spun-bonded polyester, allows it to grow healthily (Hilje et al. 2001).

The purpose of intercropping hosts is to attract whitefly from the main crop. The intercropped host acts as a trap crop and it is mostly more preferred than the main crop (Hilje et al. 2001). The trap crop should also not act as a host for any viruses which could infect the main crop. Cucumber, which is not a host of TYLCV, was used as an intercropped host with tomato in order to attract whitefly. That led to a reduction in the incidence of TYLCV in tomato (Al-Musa 1982). Mansour et al. (2012) studied the response of whitefly, *B. tabaci*, on tomato intercropped with other host plants including chilli, eggplant and okra. They found a low whitefly population in tomato when planted with eggplant and okra. However, this tactic can act adversely. For instance in Florida, when tomato was planted with eggplant as a trap crop, eggplant acted as a source of whitefly to tomato (Stansly et al. 1998).

Regarding fertilizing, nitrogen availability affects plant growth which then affects whitefly population (Hilje et al. 2001). More *B. tabaci* B biotype have been found in fertilized poinsettia plants than in unfertilized ones (Bentz et al. 1995). The greenhouse whitefly, *Trialeurodes*

vaporariorum (Westwood) population developed more quickly on tomato plants fertilized with a high nitrogen level than a control or low nitrogen (Jauset et al. 2000).

Mulches, such as sawdust, straw and rice husk, are used to interfere with visual-finding of the host (Hilje et al. 2001) and also living ground covers are used to reduce the insect's ability to find the crop. Living covers such as perennial peanuts (*Arachis pintoi*, Fabaceae), tropical chickweed (*Drymaria cordata*, Caryophyllaceae) and coriander (*Coriandrum sativum*, Umbelliferae) were used under tomato plants (Hilje and Stansly 2008). They resulted in a reduction in the number of incoming whitefly adults, delaying the onset of tomato yellow mottle virus (ToYMoV), and a decrease in disease severity, resulting in higher yields. Similar findings were obtained when living mulches including buckwheat (*Fagopyrum esculentum* Moench) and yellow mustard (*Sinapis alba* L.) were planted with zucchini, *Cucurbita pepo* L. (Hooks et al. 1998).

The type of irrigation used can affect the whitefly population (Castle 2001). Gencsoylu et al. (2003) noted that the number of nymphs was higher in the furrow irrigated cotton fields than in the drip irrigated fields. An increase in irrigation frequency of cotton plants led to a reduction in water stress, thus a reduction in whitefly population (Flint et al. 1995).

2.4.2. Host plant resistance

Host plant resistance is one of the important components of IPM. The use of varieties resistant to insect pests has been used frequently (Nombela and Muniz 2010). There are two types of plant resistance: natural and induced. Natural resistance refers to the presence of multiple germplasm in a species which keeps a high level of resistance. The induced one is acquired after the plant is attacked (Stout et al. 2002).

An example of natural resistance is the wild tomato variety, *Solanum pennellii* (Corr.) D' Arcy (Solanaceae), Byrne and Bellows (1991) suggested that a specific gene-for-gene defense response could be effective in producing resistance against piercing-sucking insects such as aphids and whiteflies and root-knot nematodes (Fernandes 1990). The *Mi-1* gene exists in the tomato wild variety *Solanum peruvianum* L. (Smith 1944). A study of the resistance of different tomato cultivars with and without *Mi-1* gene found a lower infestation level of *B. tabaci* in the cultivars with *Mi-1* gene (Nombela et al. 2000). Induced or acquired resistance can be localized (LAR), at the site of

initial inoculation, or systematic acquired resistance (Eamsobhana et al. 2009), which occurs within the tissue away from the initial inoculation site (Agrawal et al. 1999).

A study by Nombela et al. (2004) has been conducted to determine whether resistance against *B. tabaci* could be induced in susceptible tomato cultivars after an infestation by another insect, such as aphids. They found that 3 days of infestation by 20 wingless adults of the potato aphid *Macrosiphum euphorbiae* (Thomas), Hemiptera: Aphididae, were enough for the tomato plants to acquire resistance to *B. tabaci*.

2.4.3. Biological control of *B. tabaci* B biotype

Biological control methods have proven a promising control of whitefly, *B. tabaci*. Two important reasons could give the priority to use of biological control strategies against *B. tabaci*. Firstly there is insecticide resistance of *B. tabaci* to most of the conventional insecticides (Naranjo et al. 2004; Byrne et al. 2010) and secondly the main life stages of *B. tabaci*, such as eggs and nymphs are immobile and thus vulnerable to attack (Lee et al. 2011). The natural enemies include predators, parasitoids (Gerling et al. 2001; Arno et al. Chapter 15, 2010) and fungi (Faria and Wraight 2001).

Several aphelinid parasitoids perform as important limiting factors in the population dynamics of whitefly (Gerling 1990; Karut and Naranjo 2009; Pickett et al. 2013). There are 46 *Encarsia* and 21 *Eretmocerus* described parasitoid species attacking *Bemisia tabaci* (Arno et al. 2010). The *Encarsia* spp. are endoparasitic in that their eggs are laid inside whitefly nymphs. They prefer to attack the third and fourth nymphal instars (Gerling 1990). Among *Encarsia* spp., *E. formosa* Gahan, *E. bimaculata* Heraty and Polaszek and *E. sophia* Girault and Dodd are more studied against *B. tabaci* (Gerling et al. 2001; Arno et al. 2010).

Eretmocerus spp. are key parasitoids attacking whitefly worldwide. They are parasitoids only of whitefly and act as ecto- and endoparasioids (Gerling 1990; Urbaneja and Stansly 2004; Urbaneja et al. 2007). The most common species that have proven promising against *B. tabaci* are: *E. mundus* Mercet, *E. eremius* Howard and *E. queenslandensis* Naumann and Schmidt (Stansly et al. 2005; Arno et al. 2010).

More than 150 described arthropod species belonging to 9 orders and 31 families are considered as predators of *B. tabaci*. Those predators mainly belong to Coleoptera, Heteroptera, Neuroptera, and Phytoseiid mites. Two ladybird beetles (Coleoptera: Coccinellidae) are common predators of whitefly *Serangium parcesetosum* Sicard and *Delphastus catalinae* Horn. Most of the heteropteran

predatory species belong to the families Miridae and Anthocoridae. For example, *Orius laevigatus* (Fieber) and *O. majusculus* (Reuter) consume all stages of whitefly (Arno et al. 2008). *Amblyseius swirskii* Athias- Henriot is the main phytoseiid mite that preys on whitefly especially eggs and first instars; crawlers (Arno et al. 2010).

Entomopathogenic fungi could regulate insect populations well. Most of these fungi do not need to be ingested; they directly penetrate the cuticle (Goettel et al. 2005). The most studied entomopathogenic fungi that attack *Bemisia tabaci* are *Beauveria bassiana* (Bals.-Criv.) Vuill (Clavicipitaceae), *Verticillium lacanii* (Zimmerman) (Plectosphaerellaceae) and *Aschersonia aleyrodis* (Webber) (Clavicipitaceae) (Faria and Wraight 2001). Combinations of entomopathogenic fungi with parasitoids enhance the effectiveness of the biological control strategies of suppressing *B. tabaci* populations (Arno et al. 2010).

2.4.5. Chemical control of *B. tabaci*

2.4.5.1. Conventional insecticides

Chemical control is one of the main methods used against whitefly (Belay et al. 2012). Conventional insecticides are one example of chemicals used. Examples are organophosphate and carbamate groups which both have the same mode of action that inhibiting acetyl cholinesterase enzyme, whereas the pyrethroid group acts as neurotoxic insecticides that modulate sodium channel (Palumbo et al. 2001). Conventional insecticides are often used during one season and, sometimes, used as the first option in controlling agricultural pests such as whiteflies. The efficiency of chemical control relates in part to whitefly density. When the density is low, the insecticide efficiency is more likely to be high (Ahmed et al. 2002).

Ahmed (2007) has studied the effectiveness of several mixtures of organophosphate and pyrethroid insecticides against *B. tabaci*. Potentiation and antagonism effects can result from mixing different insecticides. When ethion (organophosphate) was mixed with all pyrethroids and tested against whitefly, it showed a good potential effect. On the other hand, an antagonism was obtained when profenofos (organophosphate) was mixed with cypermethrin, bifenthrin and λ – cyhalothion. Chloropyrifos (organophosphate) was also antagonistic with cypermethrin.

Other groups of insecticides called neonicotinoids have been developed (Palumbo et al. 2001; Tomizawa and Casida 2005; Jeschke et al. 2011). They act as agonists of the nicotinic acetylcholine receptor (nAChR) (Tomizawa and Yamamoto 1993). The main examples of neonicotinoids are imidacloprid, thiamethoxam, thiacloprid, nitenpyram, acetamiprid, clothianidin and dinotefuran (Jeschke et al. 2011).

Neonicotinoids affect whitefly systemically, either by foliar spray or soil drenching. However, foliar treatment is less preferred because of the problems with achieving adequate canopy coverage and its interference with natural enemies (Byrne et al. 2010). It is clear that neonicotinoid insecticides have succeeded in the agrochemical market. After 17 years from the first commercial uses of imidacloprid, neonicotinoids now comprise 24% of the market (Jeschke et al. 2011). Imidacloprid has been used worldwide and is proven to be effective against homopteran agricultural pests including whitefly (Palumbo et al. 2001; Byrne et al. 2003).

Insect growth regulators (IGRs) like pyriproxyfen and buprofezin are a third generation of insecticides. Their mode of action is to interrupt the whitefly life cycle. Pener and Dhadialla (2012) have reviewed the insect growth regulators in detail. There are three main classes of IGRs: juvenile hormone analogues, chitin synthesis inhibitors and ecdysone agonist insecticides. Recently, *B. tabaci* developed resistance to pyriproxyfen (Tabashnik and Carriere 2007) and buprofezin (Fernandez et al. 2009).

Other insecticides, which belong to different classes, are also used against whitefly including diafenthiuron, pymetrozine, spiromesifen and spirotetramat. Diafenthiuron is a thiourea derivative with insecticidal activity against hemipteran insects (Steinemann et al. 1990). It affects the insect respiration system by inhibiting the oxidative phosphorylation and disruption of mitochondrial ATP synthesis (Ruder et al. 1991). Pymetrozine is an azomethine pyridine. It is a systemic insecticide (Flückiger et al. 1992) and affects the nerves controlling the salivary pump causing sudden stopping of feeding (Kayser et al. 1994).

Spiromesifen and spirotetramat both belong to the ketoenol group. Spiromesifen is a derivative of spirocyclic tetromic acid. It inhibits the lipid biosynthesis that relates to the development of eggs and nymphs and reduces female fecundity of *B. tabaci* (Nauen et al. 2005; Kontsedalov et al. 2009). Spirotetramat is a derivative of spirocyclic tetramic acid. It has a systemic insecticidal activity. It has been used against *B. tabaci* and it showed effective results against immature stages and a fertility reduction of females (Liu 2004; Brück et al. 2009).

Surfactants are used as wetting, spreading, emulsifying and sticking agents to enhance pesticide effectiveness. They also have insecticidal activities. For example, insecticidal soaps and mineral

oils have been used against whitefly. They are effective and can cause more than 95% mortality but phytotoxicity has been recorded in young leaves of tomato (Liu and Stansly 2000). Glucosides are examples of nonionic surfactants. They could have an efficient insecticidal activity on soft bodied insects such as whiteflies, aphids and mealybugs.

2.4.5.3. Biopesticides

The term "biopesticide" is composed of two essential parts, biological and pesticides. Several books and reviews have been published regarding biopesticides and their types (Copping and Menn 2000; Sudakin 2003; Gonzalez-Coloma et al. 2010; Bailey et al. 2011; Sporleder and Lacey 2012). Sudakin (2003) defines the biopesticides as pesticides that are derived from natural materials from animals, plants or microorganisms. Biopesticides are mainly divided into three groups. They are: (1) Microbial pesticides including: viruses, bacteria, fungi and protozoa. (2) Plant – Incorporated Protectants, which refer to the production of plants with insecticidal materials incorporated genetically, such as GM plants. (3) Biochemical pesticides, such as plant extracts, plant essential oils and semiochemicals such as insect pheromones (Sudakin 2003; Bailey et al. 2011).

The advantages of the biopesticides are that they are generally low in toxicity to humans, the environment and biocontrol agents because they are often specific to target pests. Many biopesticides are also safe in terms of no residues left in crops. Another advantage when microbial pesticides are used is that microorganisms multiply in their insect hosts and persist in the environment. Regarding plant extracts and essential oils, they are not expensive when they are produced locally (Bailey et al. 2011; Sporleder and Lacey 2012). In addition, they are often can be used at the same time with other control techniques such as using pheromones and biological control and, therefore, can be incorporated into IPM programs. In organic farming, conventional pesticides cannot be used. So, the demand for biopesticides is very high (Gonzalez-Coloma et al. 2010).

The main disadvantage of microbial biopesticides is that the microorganisms can be highly specific. Therefore, correct pest identification is required. In addition, the microorganisms are influenced by environmental factors such as temperature and relative humidity (Sporleder and Lacey 2012). Biopesticides take a longer time to manage pests than conventional insecticides and need to be applied in high quantities to reach the active concentration rates (Dimetry 2012).

Microbial insecticides refer to the commercial production of entomopathogenic microorganisms. In general, there are 1500 microorganisms that affect insects adversely (Khachatourians 2009). *Bacillus thuringiensis* (Bacillaceae) (Bt, Dipel®) is the most commonly used bacterium and widely used as a microbial insecticide against a wide range of insect pests (Betz et al. 2000; Helgason et al. 2000). In addition, there are several entomopathogenic fungi produced commercially and applied against whitefly, such as *Beauveria bassiana* (Balsamo®), *Paecilomyces fumosoroseus* (Trichocomaceae) (Bemisin®) (Wraight et al. 2000) and *Verticillium lacanii* (Mycotal®) (Korolev and Gindin 1999). *Saccharopolyspora spinosa* (Pseudonocardiaceae) (Spinosad®) is an entomopathogenic product that is produced by the bacteria and is available commercially (Kalawate and Dethe 2012).

Plant-Incorporated-Protectants (PIPs) are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can take the *Cry* gene for the Bt pesticidal protein (Bt-Cry protein), and introduce the gene into the plant's own genetic material. Then the plant, instead of the *B. thuringiensis* (Bt) bacterium, manufactures the substance that destroys the pest when it feeds on the plants. The *Cry* gene is inserted into some crop plants that are in high demand, such as corn, wheat, cotton, canola, soybean, and potato crops (Hoy 2013).

Biochemical biopesticides are naturally occurring chemical substances that control pests by non-toxic mechanisms. Conventional pesticides, in contrast, are generally synthetic chemical substances that directly kill or inactivate the pest. One of the main kinds of biochemical biopesticides used these days globally is the plant essential oils which are naturally existing plant chemicals (Gonzalez-Coloma et al. 2010; Bailey et al. 2011). Historically, before the invention of the synthetic pesticides (after the 2nd World War), natural plant extracts have been used worldwide (Gonzalez-Coloma et al. 2010). Around 400 BC at Roman Empire time, pyrethrum (*Tanacetum cinerariaefolium*, Asteraceae) was used as an insecticide. In the 1600s, nicotine was extracted from tobacco leaves and applied to control plum beetles. Rotenone was first extracted from the roots of *Derris* spp. (Fabaceae) and *Lonchocarpus* spp. (Fabaceae) in 1850s (Addor 1995).

At present, several types of plant derived biochemical biopesticides have been tested against the whitefly species and shown some interesting results (Choi et al. 2003; Farghaly et al. 2009; Pinheiro et al. 2009; Ahmed et al. 2011; Aly et al. 2011; Jafarbeigi et al. 2011; Liu at al. 2014; Fanela et al. 2015; Deletre et al. 2016).

Plants essential oils and or extracts have their pesticidal properties due to the presence of different arrays of bioactive secondary metabolites such as terpenoids (limonene), phenolic compounds (tannins), alkaloids (nicotine) or glucosinolates (mustard oil) (Bailey et al. 2011; Bart 2011; Baser

and Buchbauer 2015). Although essential oils have been widely used all over the world, their use is constantly increasing because of the strong demand for pure natural ingredients to fuel the industries of flavors and fragrances, and cosmetics, and the health industry with aromatherapy and phytomedicine (Baser et al. 2007; Baser and Buchbauer 2015).

Sugar esters, also known as acyl sugars, are a relatively novel class of insecticidal compounds produced by reacting sugars with aliphatic or aromatic fatty acids (Puterka et al. 2003). Sucrose esters are safe to the environment and occur naturally in plants. Neal et al (1984) reported that natural sucrose esters, purified from the glandular trichomes of the tobacco plant, *Nicotiana gossei* (Solanaceae) detrimentally affected whitefly, *B. tabaci* and killed the immature stage. Similarly the extract of *N. gossei* (a detergent-like acylsugar) when tested was found to be effective on young and older nymphs of *Bemisia argentifolii* at very low concentrations (Liu and Stansly 1995). The insecticidal activities of natural sucrose esters against persistent and damaging whiteflies have shown that sucrose esters are a new class of bioinsecticides and should be exploited for commercial use (George et al. 1993).

As the sucrose esters are produced in the glandular secretions of trichomes of *Nicotiana* plants, their levels on those leaf surfaces are very small, being generally less than 100 μg/cm^2 (Severson et al.1991). Thus, natural plants will not likely become economical sources of millions of kilograms per year of sucrose esters to meet the demand for controlling whiteflies or aphids. Therefore, there is a need for producing biologically-active surfactants like sucrose esters which have the capacity to control whiteflies and other soft-bodied arthropod pests as bioinsecticides. Considering the importance of natural sucrose esters, scientists have recently synthesized sucrose octanoate esters (SOEs) which are belong to the organic chemical family sucrose fatty acid esters (SFAEs). SOEs are currently used to control certain soft-bodied insects (e.g., mites, aphids, thrips, whiteflies and psyllids (Puterka et al. 2003). McKenzie et al. (2005) studied different concentrations of synthetic SOEs on *B. tabaci* B biotype developmental stages. They found that the LD_{50} values for SOEs against whitefly adults, 2nd and 4th nymphal instars were 880, 686 and 1571 ppm, respectively, whereas, the LD_{50} value against eggs was 11446 ppm. However, some egg mortality occurred at the recommended application rates of 3200 – 4800 ppm.

Novel biopesticides can be developed by screening plant parts that have been previously used before the invention of the conventional pesticides. The compounds extracted from plants are identified by isolating the compounds and evaluating their activities (Gonzalez-Coloma et al. 2010). Essential oils are mainly extracted by the steam distillation method (Isman et al. 2011; Bart 2011).

2.4.6. Integrated pest management of *B. tabaci*

Integrated pest management (IPM) is the application of different control measures such as cultural, biological, chemical and other control methods to suppress a pest (Stern et al. 1959; Cuthbertson et al. 2012). Castle and Naranjo (2009) defined IPM as the use of different control methods to reduce pest status at the same time with reduction in economic and environmental costs. A model of whitefly IPM has been designed and depended on three main keys: sampling, effective chemical control tactics and avoidance (Ellsworth and Martinez-Carrillo 2001).

Against whitefly, *B. tabaci*, IPM programs mainly consist of cultural, biological and chemical control measures. These programs are categorized into two kinds according to where a crop is cultivated, i.e. in closed greenhouse or open fields. In greenhouses, biological control, using predators and parasitoids, is the essential tactic, whereas in open fields cultural control methods such as crop rotation, sanitation, changing planting and harvesting dates, watering and fertilization, are also key factors for IPM success (Stansly and Natwick 2010).

Chemical control in IPM programs should be applied as minimally as possible and in a targeted manner as a result of pest monitoring and also only where required (e.g. spot spraying). Also it should aim to use chemicals which are compatible with predators and parasitoids. The use of insecticides depends on the whitefly population density level (Stansly and Natwick 2010). For example in cotton fields, Naranjo et al. (1998) found that three to ten adults of whitefly in the fifth stem leaf from the top, is the action threshold. However, conventional insecticides could be used to protect the cotton ball from honeydew contamination (Chu et al. 1998). In order to increase the efficiency of IPM programs, biopesticides need to be included in the IPM. That will lead to reduced insecticide resistance and also reduce use of conventional insecticides.

CHAPTER 3: A preliminary study on the insecticidal effects of essential oil formulations against developmental stages of *Bemisia tabaci* B biotype

Abstract

This study assessed the effects of 30 different products and formulations against all developmental stages of the silverleaf whitefly, *Bemisia tabaci* B biotype. These included mixtures of essential oil formulations, surfactants and amines. These formulations were assessed over a range of concentrations between 0.025% v/v up to 10% v/v. A leaf dipping method was used to evaluate effects on mortality of eggs and first instar nymphs, and adults were exposed to fresh deposits of the formulations on the leaves for adult tests. Although high concentrations, 5% v/v and 10% v/v, resulted in very high mortality, phytotoxicity effects were severe. The 2% v/v of formulations showed no effects on eggs and an average mortality of 97.6% against 1st instar nymphs and 56.3% against adults. There was no effect on eggs at lower concentrations. All surfactants and formulations showed high effects on the 1st nymphal instar of *B. tabaci* B biotype. Adult mortality of surfactants and formulations showed an increase from 32% to 56.3%, respectively at 2% v/v. Some essential oils such as *L. petersonii* and *L. myrtle* showed severe phytotoxicity effects at very low concentration; 0.01%. However, there were no effects when they tested at 0.005% against SLW eggs. Three tested amines caused adult mortalities between 90% and 95.2% even at 0.5% v/v. However; these amines had no effect on eggs and nymphs. A mixture of mustard oil (75%) and liquid soap (25%) showed high mortality rates against eggs at 0.25% and adults at 1%. These experimental results were used to develop further replicated experiments to test effective formulations against the toxicity and behavioral disruption of the developmental stages of *B. tabaci* B biotype and its natural enemies (chapters seven, eight and nine).

Key words: *Bemisia tabaci,* surfactants, essential oils, solvents, amines, mortality rate.

3.1. Introduction

Biopesticides, such as pyrethrum and nicotine were used widely against insect pests in plant protection before the discovery of synthetic insecticides. In the twentieth century, the most likely primary resource of pesticides was of natural origin (Hassan and Gokce 2014). One example of an essential oil used in insect pest management is tea tree (*Melaleuca alternifolia*, Myrtaceae) oil, which contains various mono-and sesquiterpenes as well as aromatic compounds (Buckle 2003).

Essential oils are volatile liquids, or semi-liquids, typically forms with the complex mixtures of compounds usually obtained by steam distillation. Essential oils are often used in human food as flavours and fragrances (Coppen 1995) and in alternative medicines as antiseptics and mosquito repellents (Baser et al. 2007; Baser and Buchbauer 2015). Plant extracts that mimic the ecdysone hormone of arthropods, which affects and deforms the post-embryonic molting stages of different arthropods, could be a valuable approach to integrated pest management programs to replace some of the synthetic chemical pesticides in agriculture and vector management.

Surfactants generally are used as in personal care products (McDonnell and Russell 1999). Some, like glucosides, are commonly used as emulsifiers, cleansing agents and fragrance products. Glucosides are produced by condensation of fatty alcohols and glucose. Previously, biological activities of some glucosides against insect pests were researched, for examples stored product insect pests (Cis et al. 2006), the red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Chowdhury et al. 2011) and the green peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Saguez et al. 2013). Surfactants may affect agricultural pests by disrupting the waxy layer of insect cuticles or by enhancing the toxic effect of essential oils (Liu and Stansly 2000). The novel surfactants that were used in this study were used for the first time and there is very limited literature available about their efficacies on different arthropods. They are: capryl glucoside, decyl glucoside, lauryl glucoside and lauryl sucroside. No studies have been found in the literature about the use of these surfactants against insect pests. In these experiments some formulations containing surfactants and essential oils were tested to assess their effectiveness against SLW developmental stages. Two essential oils were used in these experiments, alpha- Tops and Eugenol.

Glycol ether solvents that are used in various formulations including paint strippers and wax removal may theoretically disrupt the whitefly waxy coat. Amines such as monoethanolamine (MEA) and diethanolamine (DEA) that are used in production of emulsifiers or detergents could affect the outer waxy layer of whitefly. The objective of these experiments was to evaluate efficacy of different surfactants and formulations against silverleaf whitefly developmental life stages using non replicated tests.

3.2. Materials and Methods

3.2.1. Whitefly and host seedlings (cotton, sweet chilli and tomato)

Silverleaf whitefly, *Bemisia tabaci* B biotype was initially obtained from a colony reared in the Department of Agriculture and Fisheries (DAF) laboratories at the Leslie Research Centre in Toowoomba (QLD). It was reared on cotton seedlings in 45 X 45 X 45 cm cages in an insectary in the University of Queensland, Gatton Campus maintained at 27 ± 1°C, RH 60±10%, and 14:10 (Light: Dark) photoperiod. The cotton seedlings were watered regularly. Adults were kept to lay eggs. The eggs hatched in approximately 7 days. Nymphal stage was completed in approximately 15 days and the duration of the pupal stage was approximately 6 days.

Cotton seeds were also obtained from the DAF Leslie Research Centre. The cotton variety was Sicot 71RRF, which is a Bollgard II Round up – ready variety. Three seeds were sown in 1.5 liter plastic pots using potting media. Tomato seedlings, *Lycopersicum esculentum* (Solanaceae) variety Grosse Lisse, and sweet chilli, *Capsicum annuum* (Solananceae) variety Capsicum Sweet were obtained from a local nursery in Gatton. Two seedlings were transplanted in 1.5 liter plastic pots using potting media. The media consisted of composted pinebark and woodchips. Nutrients added to the media include Osmocote Exact (3-4 month release NPK), Osmocote Plus (8-9 month release NPK), Nutricote (7 month release NPK), Osmoform (4 month release NPK), coated iron (28% iron and 17% sulphur), Saturaid (granular wetting agent) and dolomite. The seedlings were grown under glasshouse environment. The seedlings were watered regularly using an automatic watering system. Seedling leaves were used for leaf dipping and spraying bioassays.

3.2.2. Surfactants and essential oils

3.2.2.1. Test 1

Knowledge gaps exist in this area regarding the effects of the surfactants on the SLW life cycle. Initially, four surfactants were tested alone to determine their effects against all SLW developmental stages. They were:

- Capryl glucoside
- Decyl glucoside
- Lauryl glucoside
- Lauryl sucroside

Preliminary non-replicated tests were conducted at different concentrations of the surfactants (0.2% v/v, 0.4% v/v, 0.6% v/v, 0.8% v/v, 1% v/v, 2% v/v, 5% v/v and 10% v/v), tested against all developmental stages of silverleaf whitefly to determine the proper effective concentrations that

then were mixed with essential oils. From the preliminary tests, higher concentrations (>2%) showed severe phytotoxicity effects, leaves burned and severely dried. The lower concentrations (<0.5%) showed no effects on eggs and adults but high mortalities against nymphs. Therefore, concentrations; 0.5% v/v, 1% v/v, 1.5% v/v and 2% v/v were used to test the efficacy of surfactants against nymphs and adults of the silverleaf whitefly. Then lower concentrations, 0.025% v/v, 0.05% v/v. 0.125% v/v 0.25% v/v and 0.5% v/v were used against nymphs to determine LD_{50} and LD_{90}.

3.2.2.2. Test 2

A group of essential oils which are industrially purified from the following plants were tested against SLW eggs using sweet capsicum, *Capsicum annuum* leaves as the test substrate. They were:

- Clove, *Syzygium aromaticum* (L.) Merr. and L.M.Perry bud oil (CBO)
- Lemon-scented Tea Tree, *Leptospermum petersonii* leaves oil (LSTO)
- Lemon scented myrtle*, Backhousia citriodora* F.Muell. levaes oil (LMO
- Gamma tops (gamma terpinene and alpha terpinene)(GTO)

Firstly, they were tested for phytotoxicity effect using different concentrations 2%, 1.5%, 1%, 0.5%, 0.25%, 0.1%, 0.05%, 0.025% and 0.01%. Then, 0.01% and 0.005% were used to assess the egg mortality.

3.2.2.3. Test 3

Formulations containing 70% surfactants and 30% essential oils were used in experiments. The formulations were:

- Capryl glucoside and alpha- Tops (CG1)
- Capryl glucoside, alpha- Tops and 25% eugenol (CG2)
- Capryl glucoside, alpha- Tops and 37.5% eugenol (CG3)
- Capryl glucoside, alpha- Tops and 50% eugenol (CG4)
- Lauryl glucoside and alpha- Tops (LG1)
- Lauryl glucoside, alpha- Tops and 25% eugenol (LG2)
- Lauryl glucoside, alpha- Tops and 37.5% eugenol (LG3)
- Lauryl glucoside, alpha- Tops and 50% eugenol (LG4)

All products were supplied by BioAust Pty Ltd (Jimboomba QLD) were tested at 2% against the developmental stages of SLW.

3.2.2.4. Test 4

Seven surfactants were assessed against egg and adult stages of SLW. They were:

- Diethylene glycol monomethyl ether (Cellosolve acetate), DEGME (100%)
- Diethylene glycon monomethyl ether, DEGME1 (100%)
- Diethylene glycol monobutyl ether, DEGBE (100%)
- Diethylene glycol monobutyl ether acetate, DEGBEA (100%)
- Laureth – 7ethylene oxide-carboxylate as the sodium salt, LEOCS (30%)
- Laureth – 7ethylene oxide-carboxylate as the triethanolamine salt, LEOCT (30%)
- Short chain polyglucoside, SCPG (50%)

These solvents were first tested against eggs and adults at 0.5% v/v. These resulted in no effect on eggs. DEGBE, DEGBEA, LEOCT and SCPG resulted in adult mortality greater than 40%. These ones were tested again against adults using different concentrations: 0.5%, 1%, 1.5% and 2%.

3.2.2.5. Test 5

After experimenting with seven different surfactants in section 3.2.2.4, five amine products were also tested against eggs and adults to establish efficacy rates and to calculate egg and adult mortality rates. The amines were:

- Monoethanolamine (MEA)
- Diethanolamine (DEA)
- Triethanolamine(TEA)
- Monoisopropanolamine (MIPA)
- Diisoprpanolamine (DIPA)

There were first tested at 0.5% and 2%. At 2%, phytotoxicity was severe, therefore tested rates were reduced to 0.025%, 0.05%, 0.1%, 0.25%, 0.5% and 1% to establish the efficacy rates without phytotoxicity to the plants.

3.2.2.6. Test 6

In Oman, Prof. Dr. Nabil Abdel Salam, Plant Protection Expert in the Royal Court Affairs (Salalah) tested a formulation containing 50% of mustard oil and 50% liquid soap against whitefly adults. This formulation was named as Naboil50%. The results of Naboil50% at 0.5% v/v were shown to be very effective against whitefly adults (Personal communication, Abdel Salam). According to that, experiments were conducted against all the developmental stages of silverleaf whitefly under laboratory conditions. Mustard oil mixtures (mustard oil 50% and mustard oil 75%) tested at 0.25% and 1% against SLW eggs and adults.

3.2.3. Mortality test procedures

One day before the experiment, cotton leaves were removed from seedlings and placed in 20 ml plastic tubes filled with deionized water. The next day, 15 adults were aspirated and introduced to each leaf into a clip cage (2 cm in diameter) where they deposited eggs (Figure 3.1). After 24 - 48 h, adults were then removed. Thirty eggs were counted and the leaf beside each egg marked under dissecting microscope with a water proof pen.

Figure 3.1: Clip cages used for testing the biological parametes such as mortality rates in no-choice assays.

Solutions were then prepared using the following formula:

$V_1C_1 = V_2C_2$

$V_1 = (V_2C_2)/ C_1$

Where;

V_1 = volume of formulation,

C_1 = concentration of formulation,

V_2 = total volume of prepared solution,

C_2 = concentration of prepared solution.

For tests against eggs, the leaves containing marked freshly eggs were dipped for 5 sec. in the prepared solutions and left to dry then placed in the 20 ml plastic tube filled with water for 10 days until egg hatching was completed. Egg hatching percentages were observed over 10 days after egg laying (n=110, Figure 3.2). Egg mortality percentage was calculated by counting unhatched eggs multiplied by 100 then divided by the total number of eggs deposited.

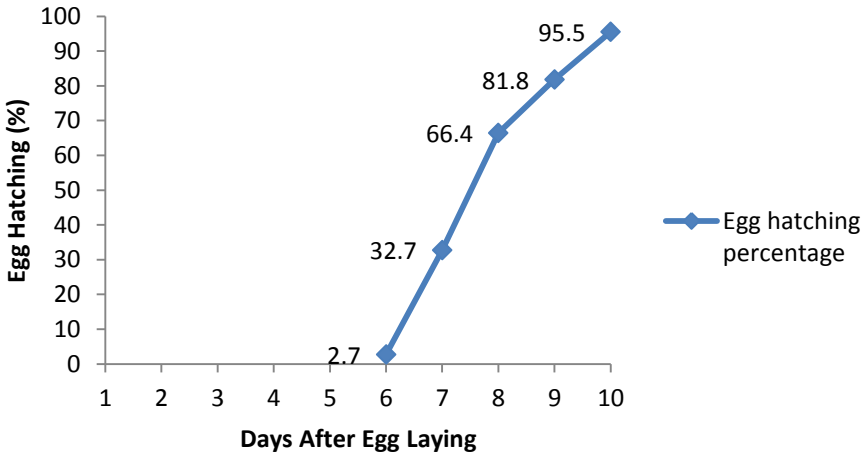


Figure 3.2: SLW egg hatching percentages during 10 days after egg laying.

For the nymphal stage tests, after the adults were removed from clip cages, the eggs were kept for 10 days to hatch. One day later, all 1st nymphal instars were left to settle on the leaf where insert their mouthparts in the cells and become sedentary and then counted and marked. A dipping method was used as in the egg experiment. Nymphal mortality percentages were calculated after 5 days.

In adult tests, 30 adults (15 males and 15 females) were introduced into each clip cage. Leaves were sprayed, then immediately, the adults were exposed to the sprayed leaves. Mortality percentages were calculated 24 hours after adult introduction. Eggs per female per day were counted after 72 hours. Another trial was performed in which leaves were sprayed and allowed to dry for 2 hours before adults were introduced, however there were no effects of any formulations and therefore this was discontinued.

High concentrations showed adverse effects on leaves. Phytotoxicity effects were also noted, categorized as low (10%), mild (20% - 30%), moderate (40% - 50%) and severe (>60%). The phytotoxic symptoms included tip and marginal burning, necrotic spots and curled leaves.

3.2.4. Scanning electron microscope for SLW eggs

Scanning electron microscope (SEM) was used to determine whether the outer surface of the SLW egg is smooth or sculptured. Taking images was according to the following procedure. Egg samples were fixed in 3% glutaraldehyde in 0.1M sodium cacodylate buffer, washed in 0.1M sodium cacodylate buffer in a Pelco® Biowave processor (Ted Pella Inc) at 250W, then secondary fixed in 1% osmium tetroxide in 0.1M sodium cacodylate buffer in the biowave processor under vacuum at 80W before being dehydrated in a series of ethanols in the biowave at 250W. They were then critically point dried in an Autosamdri critical point dryer (Tousimis), mounted on double-sided carbon tabs on aluminum stubs and coated with gold in an SPI sputter coater before viewing in a JCM 5000 Neoscope™ Table Top SEM (JEOL) operating at 10kV.

3.3. Results

3.3.1. Test 1

When high concentrations (5% and 10%) of all surfactants were tested, phytotoxicity effects were severe as shown in figure 3.3. The surfactants lauryl sucroside (LS), lauryl glucoside (LG), capryl glucoside (CG) and decyl glucoside (DG) caused different mortality rates on *B. tabaci* B biotype developmental stages. When these surfactants were tested against eggs, there were no significant effects.

Figure 3.3: Severe phytotoxicity effects of the surfactants at 5% v/v and 10% v/v. The leaves were completely burned.

Figures 3.4 and 3.5 show the mortality rates of the four surfactants tested against the first nymphal instar. The efficacy reached 100% mortality rates at concentrations between 0.25% v/v and 2% v/v. When the concentrations were reduced for example at 0.125% v/v, decyl glucoside and lauryl sucroside caused high mortality (97.5%) whereas the mortality rates of capryl glucoside and lauryl glucoside were 74.1% and 85.6%, respectively. Lauryl glucoside and decyl glucoside caused high mortality rate (69.1% and 51.2%, respectively) at 0.05% v/v and 56.3% and 45.7%, respectively at 0.025% v/v. When old nymphs (third and fourth instars) were tested by the four surfactants at 0.25% v/v, the mortality rates of older instars were much less than in the younger instars (less than 10%) (Figure 3.6).

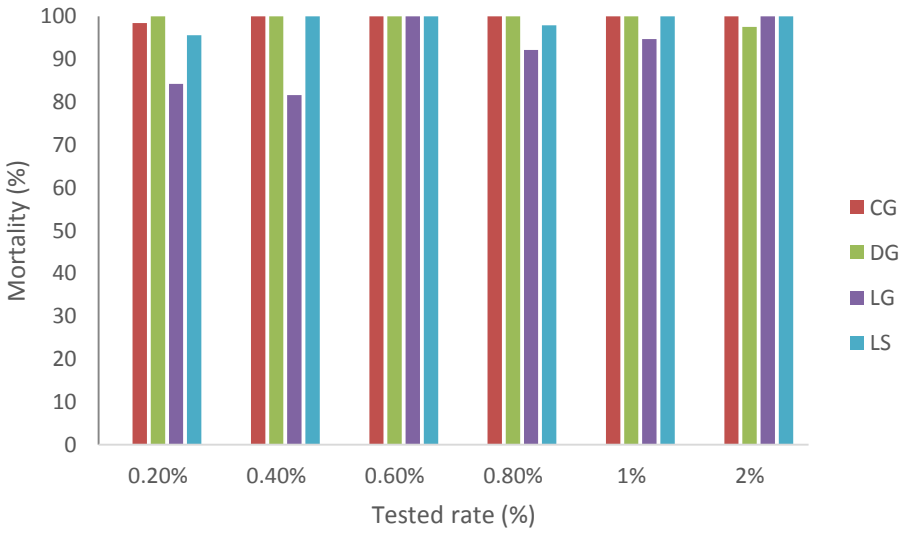


Figure 3.4: SLW 1st nymphal instar mortality of four surfactants at different concentrations (0.2% - 2%).

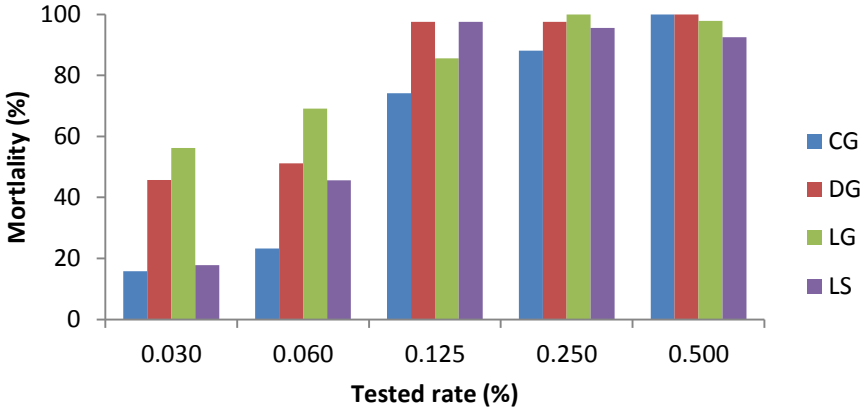


Figure 3.5: SLW 1st nymphal instar mortality of four surfactants at different concentrations (0.03% - 0.5%).

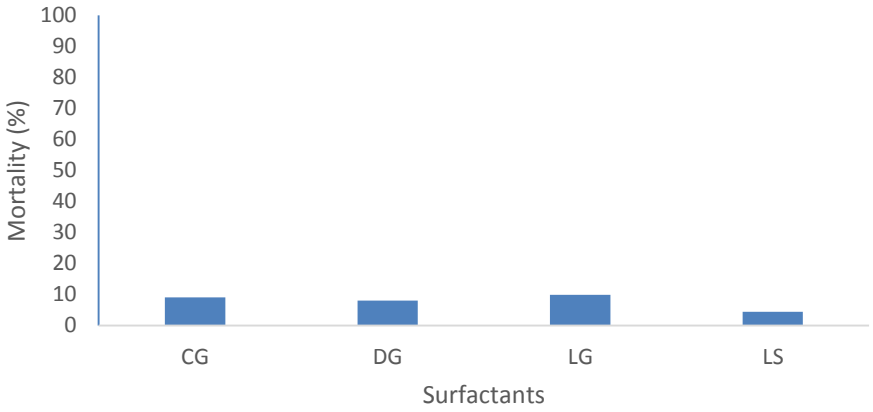


Figure 3.6: SLW old nymphal instar mortality of four surfactants at 0.25%.

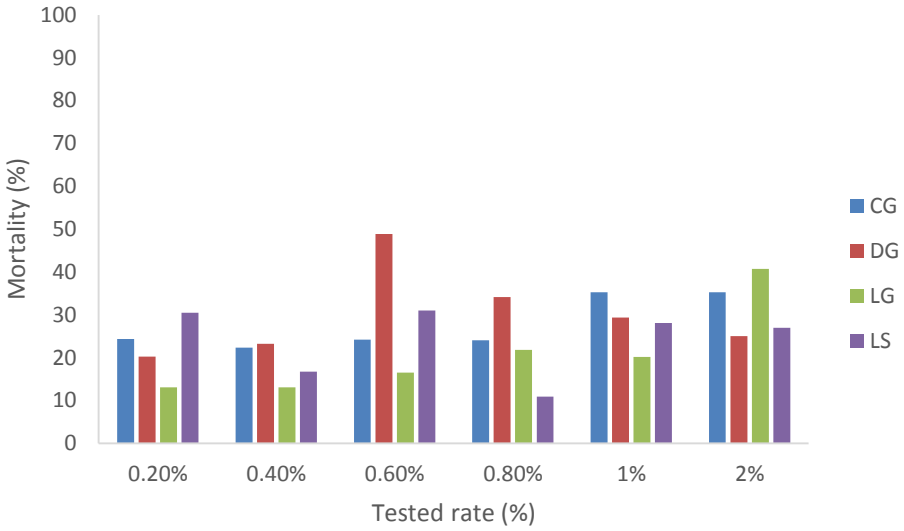


Figure 3.7: SLW adult mortality of four surfactants at different concentrations.

As can be seen in figure 3.7, all four surfactants resulted in low adult mortality at all tested concentrations. At 2%, LG, CG, LS and DG caused mortality percentages of 40.7%, 35.2%, 27% and 25%, respectively.

3.3.2. Test 2

The clove bud oil, *L. petersonii*, *L. myrtle* and gamma tops tested at concentrations between 0.01% and 2%, showed phytotoxicity effects varying from severe to mild. Clove bud oil and gamma tops showed no adverse effect on leaves at 0.01%. There were some signs of phytotoxicity effects on sweet chilli leaves of *L. petersonii* and *L. myrtle* at 0.01% and clove bud oil and gamma tops at 0.025% (Figure 3.8). When clove bud oil and gamma tops were used at 0.01% and *L. petersonii* and *L. myrtle* at 0.005% to assess the egg mortality of those essential oils, there were no effects on eggs.

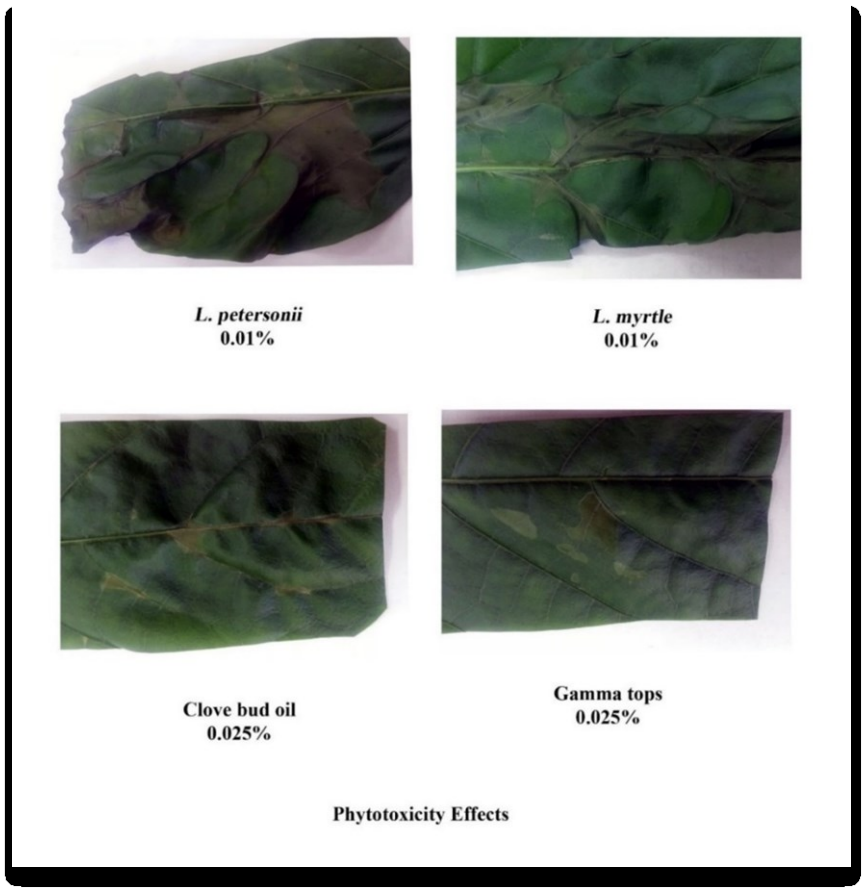


Figure 3.8: Phytotoxicity effects of essential oils at 0.01% and 0.025% on sweet chilli leaves.

3.3.3. Test 3

Eight formulations were tested separately at different concentrations between 0.025% to 10% v/v. Concentrations above 2% showed severe phytotoxicity effects on leaves, therefore, 2% was decided to be the higher concentration to be tested. The results showed that there was no effect of the formulations on SLW eggs at 2% v/v, almost all eggs had hatched 10 days after leaf dipping (DALD).

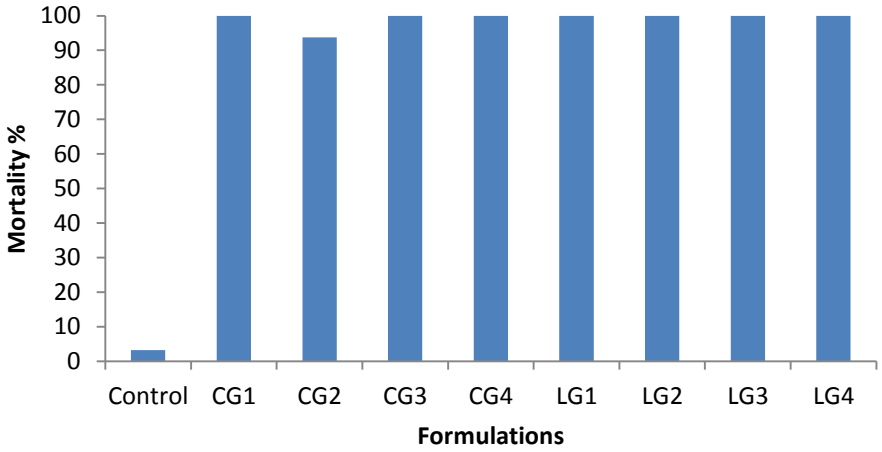


Figure 3.9: SLW young nymph mortality percentages at 2% v/v of formulations on cotton leaves.

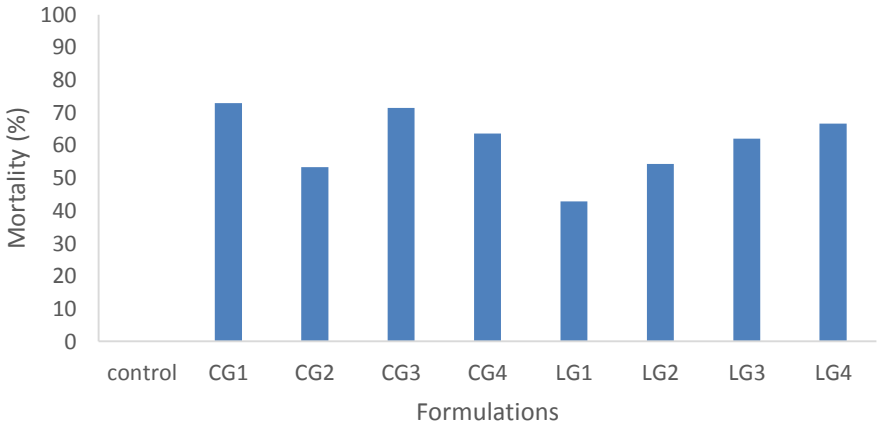


Figure 3.10: SLW old nymph mortality percentages at 2% v/v of formulations on cotton leaves.

Figure 3.9 shows the results of the mortality rates of the eight formulations against young nymphs. The nymphs which were used in the experiment were first instar nymphal stage. It is clear that all formulations had a high mortality effect on this stage reaching 93.8% to 100% comparing with a

control mortality of 0%. Figure 3.10 summarises the efficacy of the formulations at 2% against old nymphs (third and fourth instars). Old nymphs were less affected by the formulations than the younger ones with mortality between 42.8% (lauryl glucoside and alpha- Tops (LG1)) and 72.9% (capryl glucoside and alpha- Tops (CG1)).

The effect of 2% of the formulations against adults showed an average mortality rate of 56.3% (Figure 3.11). Adults were killed immediately when they were exposed to wet leaves. The live adults were left inside the clip cages and eggs counted after 72 hours. The number of eggs per female per day after treatment with the formulations is presented in figure 3.12. It showed that the number of eggs laid by surviving females in all treatments was similar to the control.

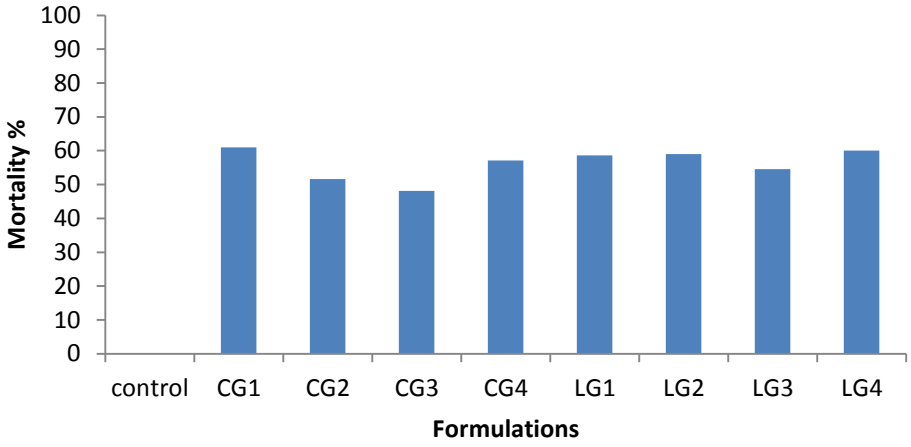


Figure 3.11: SLW adult mortality percentages at 2% v/v of formulations on cotton leaves.

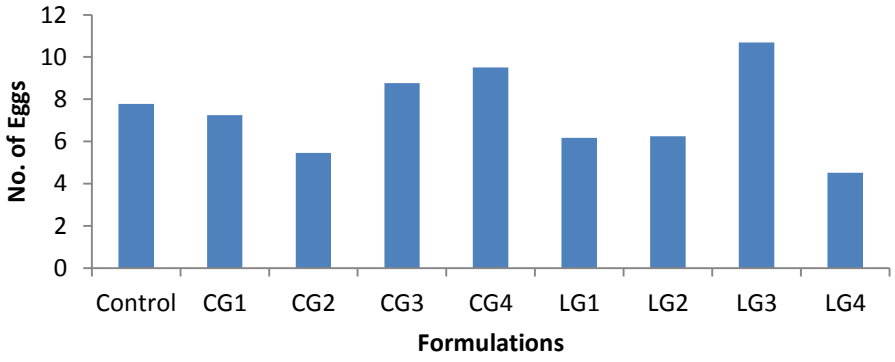


Figure 3.12: Number of eggs laid by SLW female per day after treatment with different formulations on cotton leaves.

3.3.4. Test 4

Seven water soluble surfactants were used in this test. There was no effect on eggs. When the solvents were tested against adults at 0.5% v/v, LEOCT, DEGME and DEGME1 showed the lower mortality effects as 3%, 20% and 22%, respectively. However LEOCT, SCPG, DEGBE and DEGBEA resulted in higher mortalities as compared with the other surfactants as 41.5%, 46.7%, 63.3% and 66.7%, respectively (Figure 3.13). Those four solvents were tested at different concentrations; 1%, 1.5% and 2% as shown in figure 3.14. There was no difference of the effect between the lower (0.5%) and higher (2%) concentrations for all tested surfactants. The average mortality rate was 55.1%.

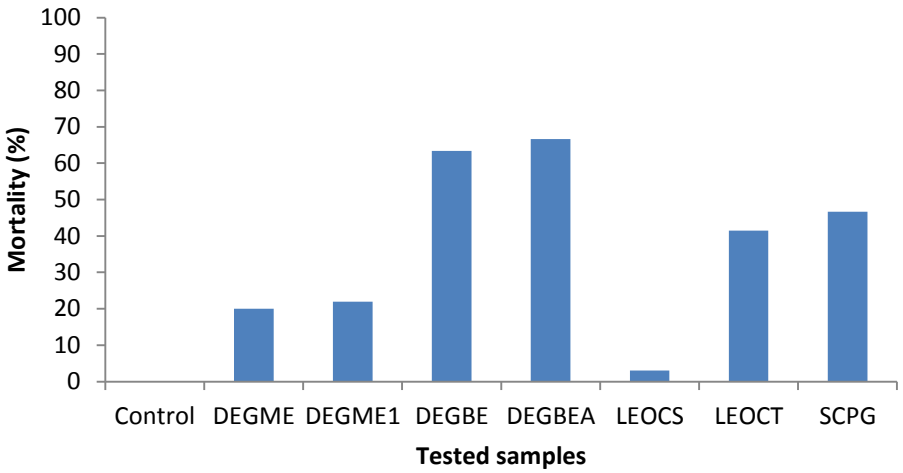


Figure 3.13: SLW adult mortality rates at 0.5% of seven samples on sweet chilli leaves.

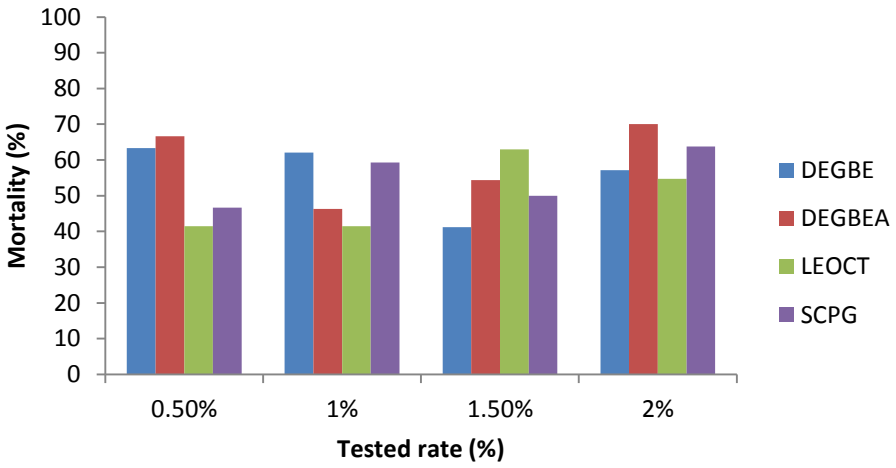


Figure 3.14: SLW adult mortality rates at different concentrations of four samples on sweet chilli leaves.

3.3.5. Test 5

The results of five amines samples are presented in figures 3.15 and 3.16. The tested amines were monoethanolamine (MEA), diethanolamine (DEA), triethanolamine (TEA), monoisopropanolamine (MIPA) and diisopropanolamine.(DIPA). Firstly, their efficacy against SLW adults was tested at the lowest concentration, 0.5%. DEA and TEA showed low mortality rates, 19.4% and 23.3%, respectively (Figure 3.15). However, MEA, DIPA and MIPA caused high mortalities on SLW adults: 90%, 90.3% and 95.2%, respectively.

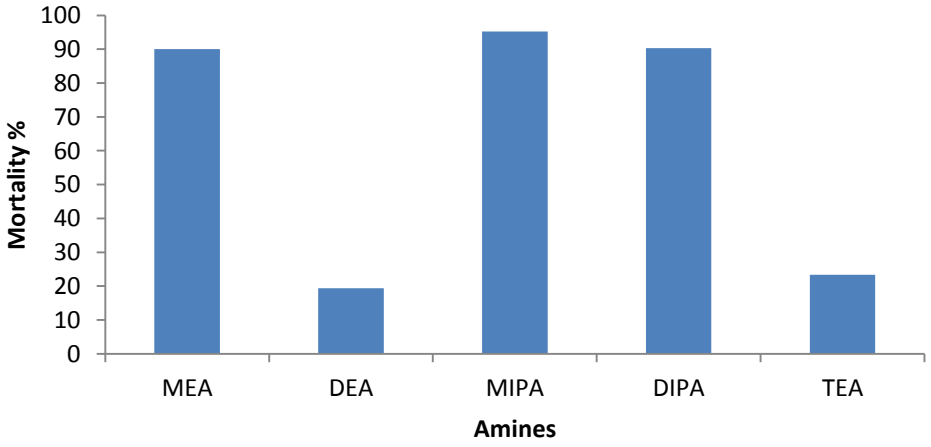


Figure 3.15: SLW adult mortality percentages at 0.5% v/v of Amines on tomato leaves.

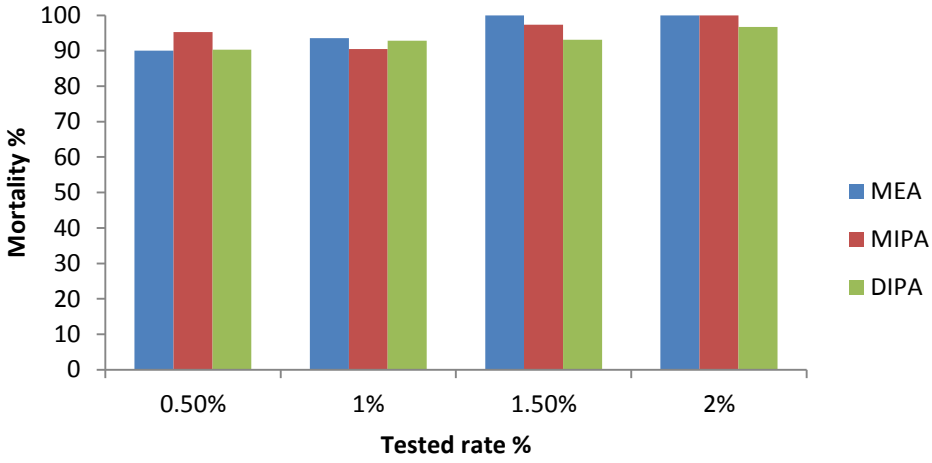


Figure 3.16: SLW adult mortality percentages at different concentrations of Amines on tomato leaves.

The effective amines; MEA, MIPA and DIPA, were then tested at different concentrations as showed in figure 3.16. All of the amines showed high mortality rates without differences between them at each concentration. The mortality rates ranged between 90% and 100%.

When three amines were tested against eggs at 0.5% and 2% using the leaf dipping method, phytotoxicity was severe at 2% and medium at 0.5%. However, there were no effects on eggs. In addition, there were no effects on first instar nymphs using either test methods leaf dipping or spraying.

3.3.6. Test 6

When mustard oil formulations (mustard oil 50% and mustard oil 75%) were tested, there were low to moderate mortality effects on adults of both formulations at both rates (0.25% and 0.5%). They were between 35% and 55%. However, both formulations resulted in high egg mortality rates at 0.5% (≈90%). Further replicated tests of the mixture of mustard oil and liquid soap against all SLW developmental stages at different rates in were presented in chapter six. Appendix 1 presents the efficacy of 30 formulations against different stages of SLW.

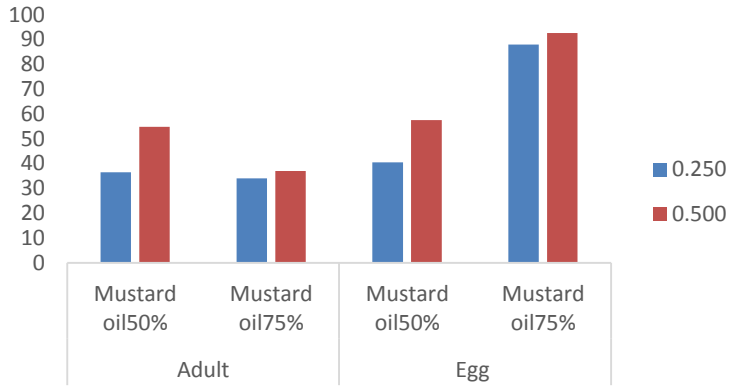


Figure 3.17: Mortality rates of two mustard oil formulations (Mustard oil50% and Mustard oil75%) against adult and egg stages at 0.25% and 0.5%.

3.3.7. Scanning electron microscope for SLW eggs

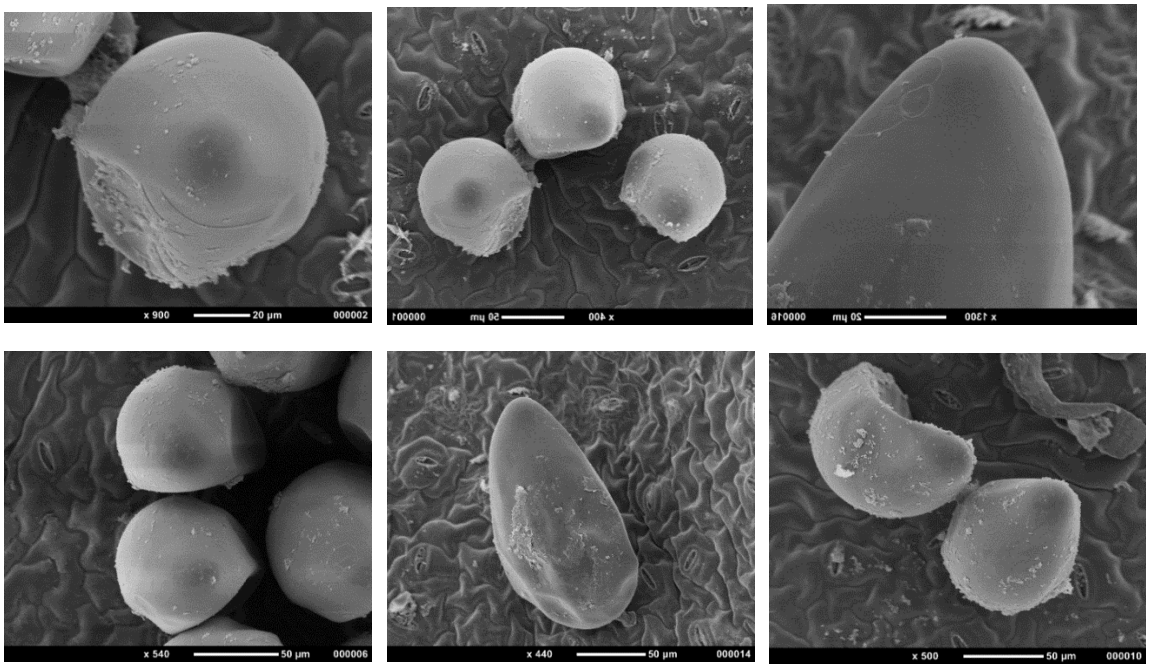


Figure 3.18: Scanning electron microscope (SEM) of silverleaf whitefly eggs showing the smoothness of egg surface.

Examination of the eggs under the microscope revealed that the egg surface was smooth (Figure 3.18).

3.4. Discussion and Conclusions

The tested surfactants are mild non-ionic surfactants. They have a contact mode of action. Phytotoxicity was the main adverse effect on cotton plants when high concentrations were used, but low concentrations had no effect on SLW eggs and a slight effect on the adult stage. However, these surfactants were very effective against nymphal stages even at very low concentrations. Some of the essential oils, such as *L. petersonii* and *L. myrtle* showed severe phytotoxicity effects at very low concentrations; 0.01% and 0.025%. However, there was no effect when they were tested against SLW eggs. Therefore, no further experiments were carried out with these essential oils.

In general, most of the tested products that include surfactants and essential oils, had no effects on eggs. Each egg has an extension of the chorion called a pedicel. Besides anchoring the egg to the host leaf, the pedicel serves as the main way through which moisture is absorbed from the host plant. In SLW, the egg pedicel is inserted directly into a slit made by the female ovipositor. During the insertion, a glue-like substance is secreted by the colleterial gland that surrounds the pedicel (Gullan and Cranston 2000). Accordingly, the SLW eggs are protected well from products that have

contact mode of action like the tested surfactants. Due to the smoothness of the surface of the egg capsule, in general any liquid pesticide will run off from the capsule toward the pedicel area. The presence of a glue-like substance produced by female during the oviposition; eliminates the absorption of any pesticide through the pedicel. However, the formulation containing mustard oil and liquid soap showed an effectiveness against SLW eggs. Further experiments on the toxicity, repellence, egg oviposition deterrent tests against all developmental stages of SLW are discussed in chapters six, seven and eight.

SLW nymphs are covered with a very thick layer of cuticular lipids, mainly long-chain (C 42 - C 64) wax esters (Buckner et al. 1999). Nymphs were very sensitive to surfactants even at very low concentrations. It is likely that the tested surfactants and formulations might disrupt the external waxy layer, resulting in desiccation and death.

There was no effect of the surfactants when the adults were introduced to the leaves which were dipped and allowed to dry for two hours. The adults were killed immediately when they were exposed to the surfactants either by spraying or dipping the leaves. However, the adults did not come into immediate contact with the wet leaves for a short time were not killed. In addition to that, the females laid eggs normally.

To conclude, there was no effect on the egg stage of most of the tested surfactants, formulations and amines even at the highest recommended dose; 2% v/v on SLW eggs. However, a mixture of mustard oil and liquid soap (Naboil50%) at ratio 1:1 at 0.5% v/v, it resulted in over 50% egg mortality. Nymphs were very sensitive to all of the surfactants. 0.125% v/v caused an average mortality rate more of 88%. 32% of adults were killed by surfactants alone whereas the mortality rate increased to 56.3% when the surfactants were mixed with essential oils at 2% v/v and when exposed immediately after spraying or dipping leaves. Amines were the most effective of the samples tested against SLW adults causing mortalities between 90% and 100%. However, there were no effects observed when these amines were tested against eggs and nymphs. Following these preliminary tests, further replicated trials were conducted for selected formulations against the SLW immature stages and selected amines against the adult stage and Naboil75% against all the developmental stages of SLW in the following chapters.

CHAPTER 4: Evaluation of Insecticidal Effects of Amines against *Bemisia tabaci* B Biotype Adults

Abstract

Amines (amino alcohols) have not previously been assessed for their insecticidal effects against agricultural insect pests. The amines used in this study were monoethanolamine (MEA), monoisopropanolamine (MIPA) and diisopropanolamine (DIPA). This study assessed their effects against the adult stage of *B. tabaci* B biotype. These amines were assessed over a range of concentrations between 0.025% v/v up to 1% v/v on tomato leaves. A spraying method was used for adult tests. The tested amines caused average mortalities between 85.8% and 89.1% at 0.5% v/v and 1% v/v, respectively. At 0.25%, MEA and MIPA caused high mortality rates, 77.8% and 82.5%, respectively, whereas DIPA caused the lowest mortality rate, 43.1%. The LD_{50} values of MEA, MIPA and DIPA were 0.14 %, 0.11 % and 0.25 %, respectively. From these results, amines could potentially be used as biopesticides against the adult of silverleaf whitefly.

Key words: *B. tabaci* B biotype, silverleaf whitefly, amines, mortality rate.

4.1. Introduction

The silverleaf whitefly, *Bemisia tabaci* B biotype has developed insecticide resistance to many conventional insecticides (Denholm et al. 1998; Ma et al. 2007; Erdogan et al. 2008; llias et al. 2012). Therefore, alternative control methods including biopesticides have assessed (Isman 2000; Pinheiro et al. 2009). In this study, amines were used for the first time to determine their effectiveness against *B. tabaci*. This study helped to select the effective amines against SLW's different developmental stages.

An amine is defined as an organic base derived from ammonia (NH3) by the replacement of one or more of the hydrogens by organic radical groups. The resultant amine is designated primary, secondary, or tertiary according to the number of hydrogens replaced (Allaby 2013). In this study three amines were used: monoethanolamine (MEA), monoisopropanolamine (MIPA) and diisopropanolamine (DIPA). MEA is mainly used in ethylene amines and imines production and in personal care products and detergents (Parmar and Burridge 2004; Elaine 2013). Besides its main use in the process of carbon dioxide removal, MIPA is also used widely in many other chemical

procedures such as an emulsifying agent, crosslinking catalyst, pigment dispersant, and corrosion inhibitor (Camacho et al. 1997). DIPA is one of the amines used as water soluble emulsifiers and neutralizers in personal care products (Stott and Kleinert 2008). The aim of this study was to evaluate the insecticidal efficacy of these three amines at different concentrations against adults of *B. tabaci* B biotype.

4.2. Materials and Methods

4.2.1. Whitefly and tomato seedlings

The methods for rearing whitefly and plant seedlings are described in chapter three section 3.2.1.

4.2.2. Amines

From a preliminary test using five amines against silverleaf whitefly adults, three of them showed high mortality rates around 90% at 0.5% v/v, whereas phytotoxicity was severe when these amines were tested at 2%. The three amines selected for further testing in this study were monoethanolamine (MEA) (C_2H_7NO), monoisopropanolamine (MIPA) (C_3H_9NO) and diisopropanolamine (DIPA) ($C_6H_{15}NO_2$) (Figure 4.1). All products were supplied by BioAust Pty Ltd (Jimboomba QLD). The amines were evaluated using replicated tests at different concentrations: 0.025% v/v, 0.05% v/v, 0.1% v/v, 0.25% v/v, 0.5% v/v and 1% v/v against the adult stage of silverleaf whitefly to determine the toxicity effects.

Monoethanolamine (MEA) Monoisopropanolamine (MIPA) Diisopropanolamine (DIPA)

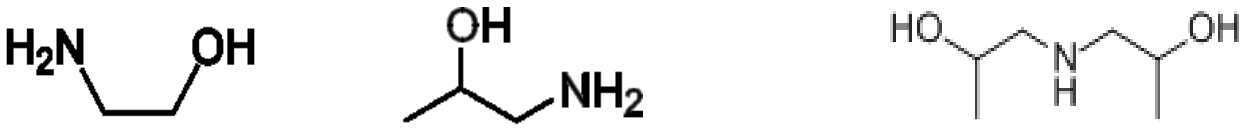


Figure 4.1: Molecular structure of the tested amines.

4.2.3. Adult Mortality test procedures

A day before the experiment tomato leaves were removed from seedlings and trimmed with a razor blade so that only the terminal leaflet remained. Each leaflet was placed in a 20 ml plastic tube filled with deionized water. The following day, 40 ml amine solutions were prepared. Then, 20 - 30 adults (males and females) were aspirated from tomato plants and introduced into a clip cage (2 cm diameter). A leaflet was sprayed thoroughly to run-off and immediately the adults were exposed to the sprayed leaflet by inserting it into the clip cage containing the adults. There were four replicates (each leaflet was considered as one replicate) for each concentration of each amine. Water was used as a control. Mortality percentages were calculated 24 hours after adult introduction. The adults were counted as dead when they remained immobile after being touched by a fine paintbrush.

4.2.4. Statistical analysis

Mortality data from all tested concentrations of amines were analyzed using Minitab 16. Probit analysis was used to estimate LD_{50} and LD_{90}. Regression analysis was used to determine relationships between percentage mortality and the tested rates of the amines. Results were assessed at the 95% confidence level. The charts were presented using the Sigma Plot program.

4.3. Results and Discussion

In literature, no previous studies have been conducted on amines (amino alcohols) against insect pests. Therefore, there was no information about their insecticidal effects. This study showed original results of three amines tested against silverleaf whitefly adults. This was the first of its kind.

From the charts in figure 4.2, all tested amines; MEA, MIPA and DIPA showed high mortalities between 82% and 95% at rates 0.5% and 1%. When the amines were tested at 0.25%, MEA and MIPA were still effective with no phytotoxicity effect. Their mortality rates were 77.8% and 82.5%, respectively. However, DIPA showed the lowest mortality rate (43.1%) at 0.25%. With the decrease in tested concentrations of amines, the mortality rates also decreased to less than 40%. Figure 4.3 shows dead adults 24 h after treatment with amines.

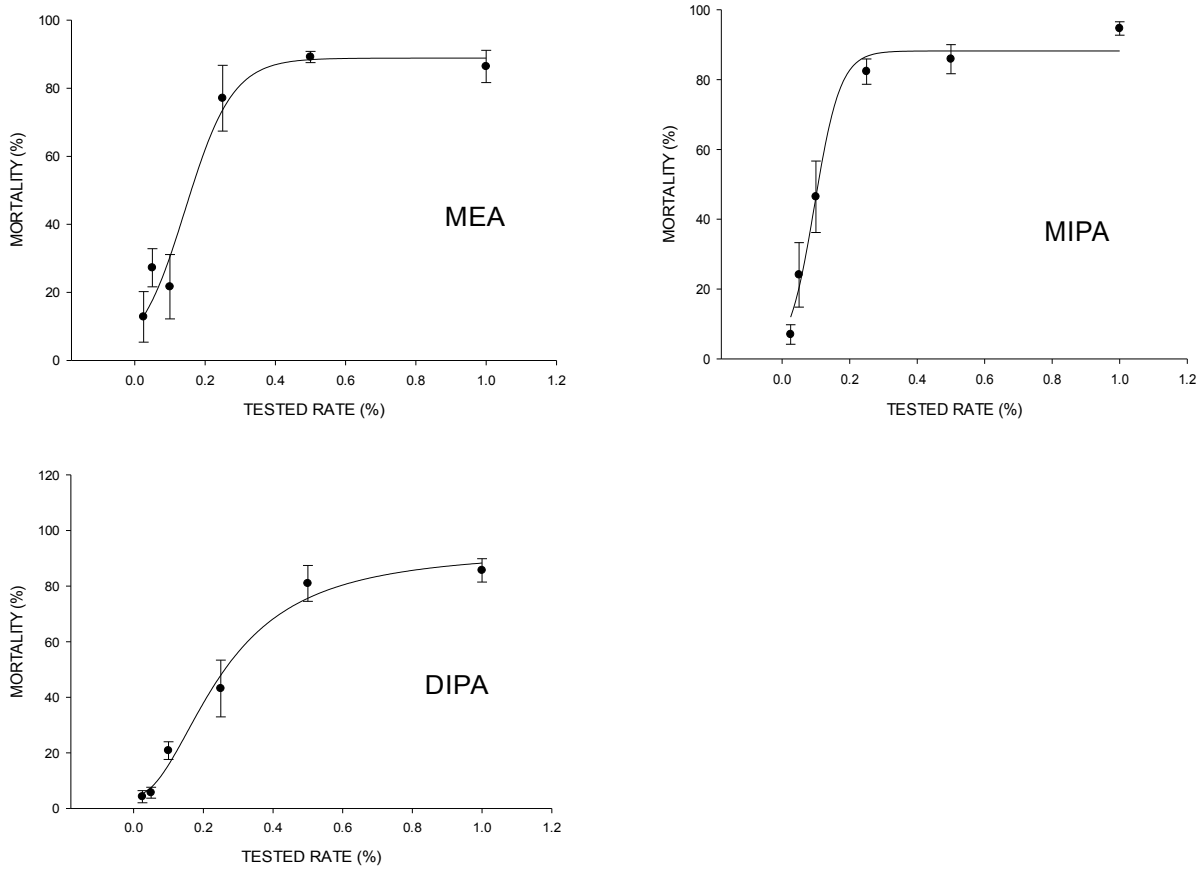


Figure 4.2: Mean mortality rates of three amines individually at different rates against SLW adult.

LD_{50} values of amines against SLW adults were calculated using probit analysis. The MEA, MIPA and DIPA were effective against adults showing LD_{50} values of 0.14%, 0.11% and 0.25%, respectively (Table 4.1). MEA showed significant effects on adults ($P < 0.001$) according to probit analysis with 95% confidence interval.

Table 4.1: Summary of toxicity of amines to *B. tabaci* B biotype adults on tomato leaves in laboratory bioassays

Amines	n	LD_{50}		LD_{90}		df	P
		(%)(± SE)	95% CI	(%) (± SE)	95% CI		
MEA	650	0.14 (± 0.011)	0.117 – 0.160	0.80 (± 0.118)	0.617 – 1.11	4	<0.001
MIPA	619	0.11 (± 0.009)	0.098 – 0.132	0.51 (± 0.066)	0.407 – 0.683	4	<0.001
DIPA	645	0.25 (± 0.018)	0.221 – 0.294	1.08 (± 0.144)	0.855 – 0.457	4	<0.001

n: number of groups tested containing 20 - 30 individuals each,
LD_{50} and LD_{90} values are in %,
SE: Standard Error,
CI: Confidence Interval,
P: Significance of fitted model.

Taken as a whole, the results showed that the SLW adults were sensitive to amines even at low concentrations. At the same time, the host plant leaves were also sensitive to the tested concentrations with phytotoxicity observed. Reducing the tested concentrations may indeed reduce mortality rates but could cause some sublethal effects including repellent, anti-feedant, and oviposition deterrent effects (He et al. 2013).

Figure 4.3: Dead SLW adults after treatment with amines

4.4. Conclusion

MEA and MIPA resulted in high mortality of adults at low concentrations, 0.25%, that did not result in phytotoxicity, whereas, DIPA caused the lowest mortality among the tested amines. These amines could play an important role in the integrated pest management programs of SLW. More research is needed to test the effects of these amines on natural enemies of SLW and, to test if they have any repellent and egg deterrent effects on adults or not. This is reported in chapters eight and nine. Results from this study will further aid to develop better and effective formulations using amines.

CHAPTER 5: Evaluation of the Toxicity and Developmental Effects of Surfactants against Nymphal Instars of *Bemisia tabaci* B Biotype

Abstract

Four surfactants, which are used in personal care products, were used in this study to evaluate their efficacy against the developmental stages of the silverleaf whitefly (SLW), *Bemisia tabaci* B biotype. These surfactants were: capryl glucoside (CG), decyl glucoside (DG), lauryl glucoside (LG) and lauryl sucroside (LS). From the preliminary tests, the surfactants showed very high mortality rates against the nymphal stage. However, they did not show good results against egg and adult stages. In this study, replicated experiments were conducted to test the mortality rates of the four surfactants against young nymphs (first and second instars) and old nymphs (third and fourth instars). The LD_{50} values of CG, DG, LG and LS against younger nymphs were 0.06, 0.04, 0.19 and 0.08%, respectively, whereas, the LD_{90} values of the same surfactants were 0.32, 0.45, 1.54 and 0.60%, respectively. High mortalities of older nymphs required high rates of the surfactants. The LD_{50} values of CG, DG, LG and LS against older nymphs were 0.81, 0.96, 0.97 and 1.04%, respectively. However, the LD_{90} values of them were 3.16, 5.71, 5.26 and 3.73%, respectively. These surfactants could be mixed with essential oils and play a promising role in suppressing SLW populations.

Key words: silverleaf whitefly, surfactants, nymphs, mortality rates,

5.1: Introduction

Surfactants generally are used as in personal care products (McDonnell and Russell 1999) such as in rinse-off and leave–on cosmetics (Krehic and Avenel-Audran 2009; Fiume et al. 2013). Some surfactants such as glucosides are commonly used as emulsifiers, cleansing agents and fragrance products. Glucosides are produced by condensation of fatty alcohols and glucose (Wei et al. 2002; Fiume et al. 2013). The biological activities of some glucosides against insect pests have been studied for example stored product insect pests (Cis et al. 2006) the red flour beetle *Tribolium castaneum* (Herbst) (Chowdhury et al. 2011) and the green peach aphid *Myzus persicae* (Sulzer) (Saguez et al. 2013). McKenzie et al. (2004 and 2005) studied the effects of SOEs against nymphs

and adults of brown citrus aphid *Toxoptera citricida* (Hemiptera: Aphididae) (Kirkaldy) and nymphs of *B. tabaci.* In the current study, initial tests of a range of glucosides found that they might have toxicity and disruption effects on the developmental stages of *B. tabaci* B biotype. They may affect agricultural pests by disrupting the external waxy layer of insect developmental stages or by enhancing the toxic effect of essential oils (Liu and Stansly 2000). The novel surfactants used in this study are used for the first time and no literature is available about the use of these surfactants against insect pests. They are: capryl glucoside, decyl glucoside, lauryl glucoside and lauryl sucroside. In these experiments some surfactants were tested to assess their effectiveness against SLW nymphal instars (Figures 5.1-5.3).

Figure 5.1: The structure of decyl glucoside.

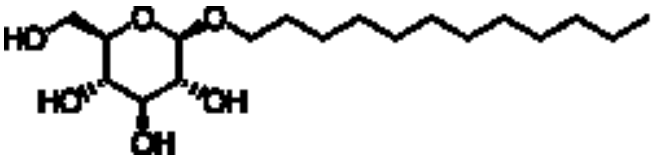


Figure 5.2: The structure of lauryl glucoside.

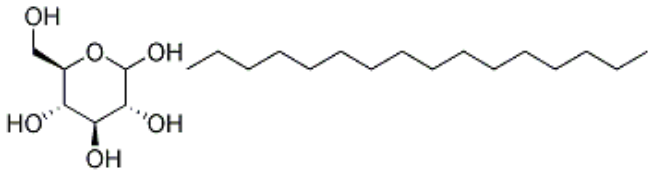


Figure 5.3: The structure of capryl glucoside.

5.2. Materials and Methods

5.2.1. Whitefly and tomato seedlings

The methods for rearing whitefly and plant seedlings are described in chapter three section 3.2.1.

5.2.2. Surfactants

Four surfactants were tested to determine their effects against all SLW developmental stages. They were: capryl glucoside ($C_{22}H_{46}O_6$), decyl glucoside ($C_{16}H_{32}O_6$), lauryl glucoside ($C_{18}H_{36}O_6$), and lauryl sucroside. Preliminary non-replicated tests (Appendix 1) were conducted at different concentrations of the surfactants, 0.025% v/v, 0.05% v/v. 0.1% v/v, 0.2% v/v, 0.4% v/v, 0.6% v/v, 0.8% v/v, 1% v/v, 2% v/v, 5% v/v and 10% v/v, against all developmental stages of silverleaf whitefly to establish effective concentrations that then were mixed with essential oils. From the preliminary tests, higher concentrations (>2%) showed severe phytotoxicity effects and lower concentrations (<0.5%) showed no effects on eggs and adults.

Therefore, concentrations; 0.5% v/v, 1% v/v, 1.5% v/v and 2% v/v were used to test the efficacy of the surfactants against the nymphs; young nymphs (first and second instars) and old nymphs (third and fourth instars) of silverleaf whitefly. At 0.5%, mortality rates of the surfactants were very high against young nymphs and therefore the concentrations were tested at reduced rates of 0.025%, 0.05%, 0.125% and 0.25%. Each concentration was replicated four times. Around 30 nymphs were tested in each replicate. Water was used as a control treatment.

5.2.3. Mortality test procedures

A day before the experiment commenced, tomato leaves were removed from seedlings and placed in 20 ml plastic tubes filled with deionized water. The following day, 15 adults were aspirated and introduced to each leaf in a clip cage (2 cm in diameter) where they deposited eggs. After 24 hours, adults were then removed from clip cages. The surfactants were tested ten days after adult removal for younger nymphs (first and second instars) and eighteen days for older nymphs (third and fourth instars). Nymphs were counted and marked under a dissecting microscope with water proof pen. After that, the leaves were sprayed with the prepared solutions and left to dry then replaced in the 20 ml plastic tube filled with water. Three days later, the mortality percentages were calculated. Shrunk and dried brown nymphs were counted as dead.

5.2.4. Statistical analysis

Mortality data from all tested rates of surfactants were analyzed using Minitab 17. Probit analysis was used to estimate LD_{50} and LD_{90}. Regression analysis was used to determine relationships between percentages of mortality and tested rates of the surfactants. Results were assessed at the 95% confidence level. The charts were presented using the Sigma Plot program.

5.3. Results and Discussion

5.3.1. SLW young nymph mortality

The graphs in figure 5.4 shows the effects of four surfactants; capryl glucoside (CG), decyl glucoside (DG), lauryl glucoside (LG) and lauryl sucroside (LS), on younger nymphs of SLW at different dilution rates. At 0.25%, mortalities of capryl glucoside and decyl glucoside were very high: 84.2% and 94.6%, respectively. Whereas, the effects of lauryl glucoside (57.9%) and lauryl sucroside (63.4%) were less than capryl and decyl glucosides. Decyl glucoside was still effective even at very low rates and the mortality of young nymphs at 0.01% was 29.9%.

LD_{50} values of decyl glucoside, capryl glucoside and lauryl sucroside showed the lowest values (0.04, 0.06 and 0.08%, respectively) compared with the LD_{50} value of lauryl glucoside, which was 0.19 %. The LD_{90} values of CG, DG, LG and LS were increased to 0.32, 0.45, 1.54 and 0.60%, respectively. Among all the tested surfactants, lauryl glucoside had the highest LD_{90} value (1.54%). DG and LS showed effects on the younger nymphs ($p < 0.001$) (Table 5.1).

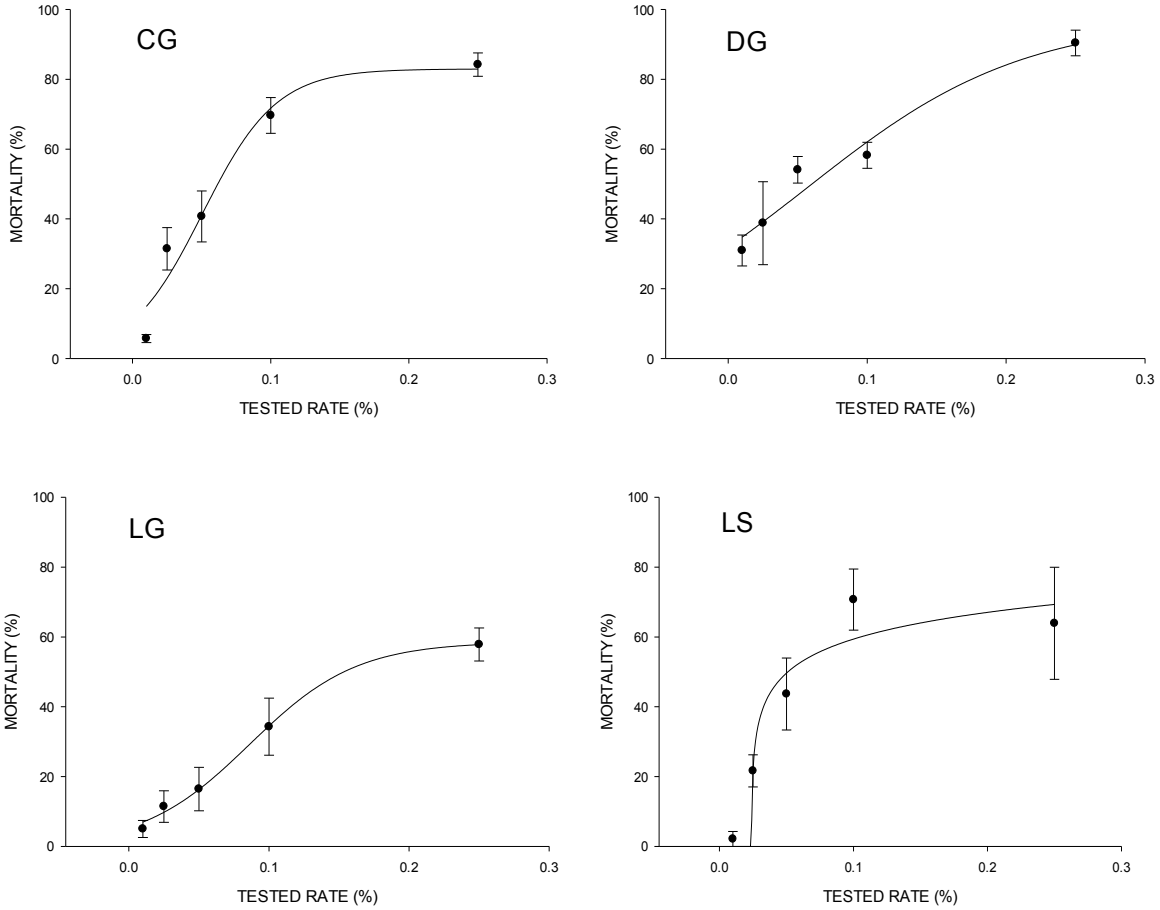


Figure 5.4: SLW young nymphal instar mortality of four surfactants at different rates.

LD_{50} and LD_{90} of the four surfactants against younger nymphs were calculated and presented in the following table 5.1:

Table 5.1: Summary of toxicity of surfactants to younger nymphs (first and second) of *B. tabaci* B biotype on tomato leaves in laboratory bioassays

Surfactants	n	LD_{50}		LD_{90}		df	P
		(%) (± SE)	95% CI	(%)(± SE)	95% CI		
Capryl glucoside	693	0.06 (± 0.004)	0.051 – 0.068	0.32 (± 0.051)	0.248 – 0.465	3	<0.001
Decyl glucoside	657	0.04 (± 0.004)	0.031 – 0.046	0.45 (± 0.113)	0.300 – 0.825	3	<0.001
Lauryl glucoside	700	0.19 (± 0.230)	0.154 – 0.251	1.54 (± 0.458)	0.938 – 3.15	3	<0.001
Lauryl sucroside	692	0.08 (± 0.007)	0.068 – 0.095	0.60 (± 0.129)	0.417 – 0.991	3	<0.001

n: number of groups tested containing 30 individuals each,
LD_{50} and LD_{90} values are in %,
SE: Standard Error,
CI: Confidence Interval,
P: Significance of fitted model.

5.3.1. SLW old nymph mortality

Figure 5.5 presents the results of the four surfactants; CG, DG, LG and LS, against older nymphs (third and fourth instars). The mortalities of older nymphs were lower than those of younger instars for the same range of rates. For example, at 0.05% and 0.1%, there were no effects of the surfactants on old nymphs and at 0.25%, the mortality rates of the four surfactants were between 8.47% and 22.5% for the older nymphs whereas the mortalities of the younger ones were between 57.9% and 94.6%. Therefore, the tested rates were increased to 0.5%, 0.75%, 1% and 1.5% to estimate the LD_{50} and LD_{90} of the surfactants. There was no big difference in mortalities of old nymphs when the surfactants were tested at 0.5%, 0.75% and 1%. The mortality rates were between 27.35% and 43.33% except for capryl glucoside. It showed higher mortality rate at 1%, which comprised 67.5%. At 1.5%, the surfactants, CG, DG, LG and LS, showed high mortalities: 73.68%, 71.67%, 80.83% and 72.5%, respectively. Table 5.2 summarizes the results of the toxicity effects against the third and fourth instar nymphal stage.

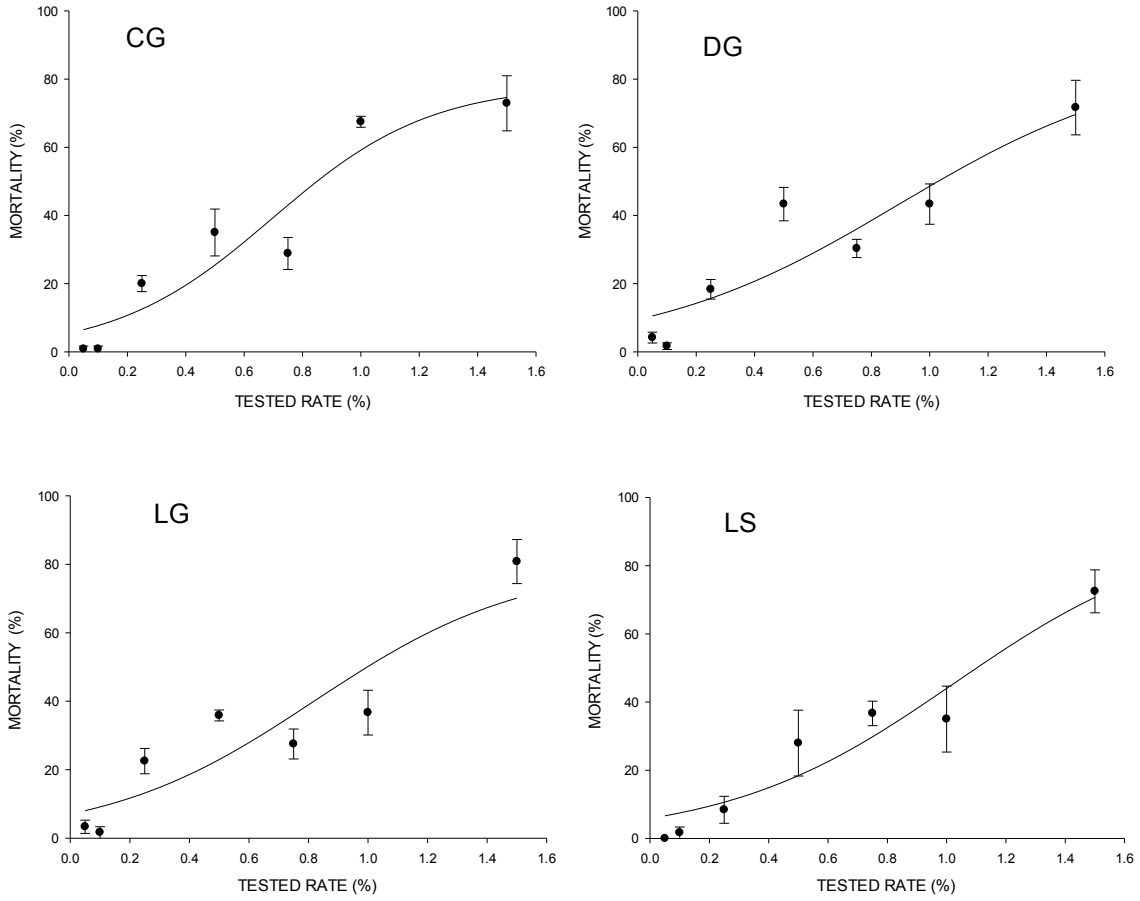


Figure 5.5: SLW old nymphal instar mortality of four surfactants at different rates.

There were no differences in the LD_{50} values between the surfactants. However, CG and LS had the lowest LD_{90} values, 3.16 and 3.73%, respectively. In comparison, DG and LG had the highest LD_{90} values, 5.71 and 5.26%, respectively. Statistically, all the surfactants showed significant effects on the older nymphs (Table 5.2).

Table 5.2: Summary of toxicity of surfactants to old nymphs (third and fourth) of *B. tabaci* B biotype on tomato leaves in laboratory bioassays

Surfactants (ON)	n	LD_{50}		LD_{90}		df	P
		(%) (± SE)	95% CI	(%)(± SE)	95% CI		
Capryl glucoside	821	0.81 (± 0.047)	0.723 – 0.909	3.16 (± 0.434)	2.496 – 4.332	5	<0.001
Decyl glucoside	839	0.96 (± 0.074)	0.830 – 1.132	5.71 (± 1.168)	4.040 – 9.22	5	<0.001
Lauryl glucoside	840	0.97 (± 0.072)	0.844 – 1.135	5.26 (± 1.019)	3.793 – 8.284	5	<0.001
Lauryl sucroside	835	1.04 (± 0.062)	0.934 – 1.187	3.73 (± 0.561)	2.893 – 5.309	5	<0.001

n: number of groups tested containing 30 individuals each,
LD_{50} and LD_{90} values are in %,
SE: Standard Error,
CI: Confidence Interval,
P: Significance of fitted model.

From the above results, it was clear that, younger nymphs were more sensitive to the surfactant than the older ones. All surfactants in this study had an effect on the nymphal stage of silverleaf whitefly, (Figure 5.6). This is the first study. There is no information in the literature about the effect of these surfactants against SLW developmental stages. However, a study by Yang et al. (2010) showed that the garden thyme, *Thymus vulgaris* L., essential oil at 0.5% reduced the survival rates of the younger and older nymphs of SLW to 79.0% and 58.2%, respectively. Our results agreed with this study that younger nymphs were more sensitive than older ones. Saguez et al. (2013) tested some glucosides against the green peach aphid *M. persicae*. From their study, the lignan, secoisolariciresinol diglucoside (SDG), and the neolignin, dehydrodiconiferyl alcohol-4-β-D-glucoside (DCG), showed a reduction in nymphal survival to at least 25% at 0.1 mg/ml. Both lignan and neolignan have been reported, in the Saguez et al. study, as having anti-feedant and deterrent properties and also a direct toxicity effect on aphids. A study by McKenzie et al. (2004) reported the toxicity of SOEs on brown citrus aphid *T. citricida* nymphs. The mortality ranged between 60% and 70% at 0.6%. Our study showed that young nymph mortalities were between 84.2% and 94.6%, respectively of capryl glucoside and decyl glucoside at 0.25% whereas mortalities of older nymphs were between 30 and 70% at 1%. When the SOEs was tested by McKenzie et al. (2005) against

whitefly nymphs, the LD_{50} and LD_{90} values for second instars were 0.07 and 0.22%, respectively, whereas those for fourth instars were 0.16 and 0.53%. We found similar LD_{50} and LD_{90} values for younger nymphs compared to McKenzie et al. whereas the LD_{50} and LD_{90} values of older nymphs were higher (1% and 3%, respectively).

Figure 5.6: The effectiveness of the surfactants on SLW nymphs (healthy nymphs – top left), (dead young nymphs – top right), (dead old nymphs – bottom left and right).

5.4. Conclusion

The surfactants, capryl glucoside, decyl glucoside, lauryl glucoside and lauryl sucroside, were tested for the first time against one of the main agricultural pests. They showed the possibility to be mixed with essential oils to form botanical product formulations to manage the immature stages of SLW. The next step is to develop new formulations in which the surfactants will be one of the main components. LG was selected for further testing and as a component of the prepared formulations tested for their toxicity and repellent and oviposition deterrent effects against SLW developmental stages and its parasitoid (*E. hayati*), presented in chapters seven, eight and nine.

CHAPTER 6: Evaluation of Insecticidal Effects of a Mustard Oil75% Formulation against the Developmental Stages of *Bemisia tabaci* B Biotype.

Abstract

Bio-pesticides are one of the main alternatives to conventional insecticides. The results of previous studies showed that some plant extracts can be used as a potential effective method to control *Bemisia tabaci* B biotype. This study assessed the effects of a mixture containing mustard oil and liquid soap against all developmental stages of SLW. This mixture was tested over a range of concentrations (0.010%. 0.025%, 0.05%, 0.1% and 0.25%) compared with a control against the egg stages of silverleaf whitefly (SLW). A different rate range was used against nymphs (0.05%, 0.1%, 0.25%, 0.5% and 1%) and against adults (0.25%, 0.5%, 1%, 1.5% and 2%). A spraying method was used for toxicity tests against all stages. For egg tests, a concentration of 0.25% resulted in 95.8% mortality whereas the mortality percentages were 43% and 50% for rates of the mixture at 0.1% and 0.05%, respectively. The LD_{50} and LD_{90} of the mixture were 0.44% and 1.73%, respectively. For nymph tests, mustard oil was effective at rates of 0.25% and above against young and old nymphs (86.4% and 47.4% mortality, respectively). The values of LD_{50} and LD_{90} were 0.13% and 0.44% respectively for young nymphs, and 0.55%, and 2.91%, respectively for old nymphs. Younger nymphs were more sensitive to mustard oil than older ones. From adult tests, at 0.25% and 0.5% mortalities were low; 34.0% and 37.0%, respectively. The mortality rate increased to 94.17% at 1%. The LD_{50} and LD_{90} of the mixture against adults were 0.42% and 1.06%, respectively. From these results, mustard oil could be used potential biopesticide against all developmental stages of silverleaf whitefly.

Key words: *B. tabaci* B biotype, silverleaf whitefly, mustard oil, liquid soap, mortality rate.

6.1. Introduction

Besides their use in food industries, plant essential oils also play an important part in other industries including the production of plastics, surfactants, inks, adhesives and pesticides (Salimon et al. 2012; Baser and Buchbauer 2015). Essential oils have been used as alternatives to the conventional synthetic insecticides against many species of insect pests (Isman 2000; Regnault-Roger et al. 2012). The *B. tabaci*, B biotype is among the agricultural insect pests that cause severe

damage to fruit and vegetable crops (Isman 2000). Many researchers have tested plant essential oils against SLW (Puri et al. 1994; Pinheiro et al. 2009; Yang et al. 2010; Yarahmadi et al. 2013; de Almeida Marques et al. 2014; Christofoli et al. 2015). Their results showed that some essential oils such as neem oil, essential oils from *Zanthoxylum rhoifolium* Lam. leaves, *Thymus vulgaris* L. and *Pogostemon cablin* Blanco have lethal and sublethal effects on the SLW developmental stages. This study aimed to look at the effects of a mixture of mustard oil and liquid soap at different concentrations.

The black mustard, *Brassica nigra* (L.) K.Koch is one of the most important oil crops which belong to the Brassicaceae family (formerly Cruciferae). Its seeds are used traditionally in medicine and food condiments (Balke and Diosady 2000; Björkman et al. 2011). Although ten thousand year old traces of cultivation give evidence that plants in the family Brassicaceae are among the oldest cultivated plants known (Snowdon et al., 2007).

Most updated reviews indicated that all economically important *Brassica* crops including the species *B. nigra* contain high concentration of sulphur containing glucosinolates (GLs)- a major class of secondary metabolites (Halkier and Gershenzon 2006). The biological activity of Brassicaceae has been thought to be due primarily to GLs and their bioactive degradation products. We know that the degradation products of GLs are produced when the cells are ruptured via mechanical damage, infection or insect attack and the GLs present in vacuoles are hydrolyzed by the enzyme myrosinase (MYR) (Rask et al. 2000; Bones and Rossiter 1996). Chemical conditions such as pH, availability of ferrous ions and presence of MYR- interacting proteins determine the final composition of the biologically active product mix which can include isothiocyanates (ITCs), oxozolidine-2-thiones, nitriles, epithionitriles, and thiocyanates (Bones and Rossiter, 1996, 2006; Rask et al., 2000). However Vaughn and Boydston (1997) when analyzed the macerated green manure leaf and stem tissues of *B. nigra* and *B. juncea* plants using gas chromatography – mass spectrometry (GC – MS) found that *B. nigra* contains 54.4% AITC and *B. juncea* contains 67.3% AITC as major compounds. The phytochemical analysis of the *B. nigra* seeds indicated the presence of saponins (12.82%), alkaloids (20.58%), flavonoids (6.57%), glycosides (20.01%, reducing sugar (5.56%), phlobatannins (15.05%) and volatile oil (25.13%) (Uzama et al. 2016).

Over the last two decades the toxicological effects of crude mustard oils, its GLs contents and their breakdown products especially ITCs which are formed after myrosinase-catalyzed hydrolysis on soft bodied insects have been of much less concern and have scarcely been investigated (Fenwick et al. 1983) compared to the intensive studies on the potential benefit on human health as anticancer agents (Cartea and Velasco 2008) and as “biopesticides” for controlling soil-borne

pathogens and against a range of pathogenic and food spoilage bacteria (Lazzeri et al. 2004; Manici et al. 1997; Wilson et al. 2013).

For example Paes et al. (2012) reported that synthetic mustard essential oil (SMEO) (90% AITC) can affect the developmental stages of the maize weevil (*Sitophilus zeamais*) (Coleoptera: Curculionidae). Similarly mustard oil from *Brassica alba* (Brassicaceae) found detrimental against the cotton leafworm, *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae) (Abd El-Aziz and Sharaby 1997). Additionally ITCs was found to be toxic against the eggs of *Dasineura brassicae* (Ahman 1985) and AITC from commercial source was also found toxic to the red flour beetle *Tribolium castaneum* as fumigant (Santos et al. 2011). Wolfson (1982) observed that developmental responses of some insects to *Brassica nigra* were due to GLs compounds. Interestingly ITCs inhibited both *in vitro* germination and subsequent growth of the insect pathogenic fungus *Metarhizium anisopliae* Sorokin (Clavicipitaceae) and its ability to infect *Phaedon cochleariae* (Coleoptera: Chrosomelidae) (Inyang et al.1999). It is positive from a plant health perspective that natural enemies may also benefit from the glucosinolate–myrosinase system in search of hosts by using volatiles, such as ITCs, emitted from infested *Brassica* plants as cues (Pope et al. 2008).

The effects of glucosinolates on insect pests have been still emphasized mainly on chemical ecological functions or role as mediators in plant-insect interactions rather than acute toxicity. In the Brassicaceae (*Brassica napus)*, for example, high concentrations of GLs and their breakdown products can serve as deterrent for generalist herbivores, while at the same time they can attract and stimulate feeding and egg laying of insects which are specialists on cruciferous plants (Giamoustaris and Mithen 1995). David and Gardiner (1966) have demonstrated that several mustard GLs have a feeding stimulation effect on the diamond-back moth (*Plutella maculipennis* Curtis) (Lepidoptera: Plutellidae) and the larvae of *Pieris brassicae* (Lepidoptera: Pieridae). The objective of this study was to evaluate the insecticidal effect of mustard oil mixed with liquid soap against the developmental stages of silverleaf whitefly, *B. tabaci* B biotype. Soap was included in the formulation as a surfactant to increase the mobility of the mustard oils across the waxy cuticular membrane of the SLW.

6.2. Materials and Methods

6.2.1. Whitefly and tomato seedlings

The methods for rearing whitefly and plant seedlings are described in chapter three section 3.2.1.

6.2.2. Mustard oil and liquid soap

A mixture was prepared from mustard oil (MSDS presented in appendix 2) and liquid soap (Trix®) (MSDS presented in appendix 3) both obtained from the local market. Both were placed in 250 mL volumetric flasks at a ratio of 3:1 (75% mustard oil and 25% liquid soap) and then mixed well. The phytotoxic effect of the mixture was tested to determine the highest concentration that did not damage the plants. 1%, 1.5%, 2%, 2.5% and 5% v/v rates were tested and showed no phytotoxic effects. The mixtures were evaluated using replicated tests at different rates (0.010%. 0.025%, 0.05%, 0.1% and 0.25%) compared with the control (water) against the egg stage of SLW. For nymphal stage tests, different rates were used (0.05%, 0.1%, 0.25%, 0.5% and 1%). Preliminary tests of the mixture at the rate of 0.25% against the adults of SLW resulted in low mortality. Therefore a higher range of concentrations were used against adults (0.25%, 0.5%, 1%, 1.5% and 2%). A spraying method was used for toxicity tests against all stages. These tested formulations were compared with the control, in this case water against the appropriate stages of SLW.

6.2.3. Mortality test procedures

A day before the experiment tomato leaves were removed from seedlings with a razor blade. Leaves were placed in 20 ml plastic tubes filled with water. The next day, 100 ml solutions were prepared from the mustard oil and liquid soap mixture and deionized water at different concentrations, dependent on the stage being tested (as described in section 6.2.2.) Formulations were applied with a low pressure hand sprayer. The leaves were sprayed until run-off.

For the egg test, 10 male and 10 female adults were introduced into clip cages (2 cm in diameter) where they deposited eggs. The adults were removed after 24 hours. Thirty eggs per leaflet were counted and a mark was put beside each egg using a water proof pen. Each leaflet was counted as a replicate and there were four replicates per test. Three days later (three day old eggs), the leaves were sprayed with the prepared solutions and left to dry then placed in the 20 ml plastic tube filled with water for 7 days until egg hatching was completed.

For the nymphal stage tests, different rates of mustard oil 75% were tested according to the methodology described in chapter five section 5.2.3.

For the adult mortality test, the method procedure described in chapter four section 4.2.3 was used.

6.2.4. Statistical analysis

Mortality data from all tested concentrations of the mustard oil and soap mixture were analyzed using Minitab 16. Probit analysis was used to estimate LD_{50} and LD_{90}. Regression analysis was used to determine relationships between percentages of mortality and tested rates of the mixture. Results were assessed at the 95% confidence level. The chart was prepared using the Sigma Plot program.

6.3. Results and Discussion

In the literature, few studies have been conducted on these formulations that consist of mustard oil and liquid soap mixture against whiteflies. In Oman, Prof. Dr. Nabil Abdel Salam (Plant Protection Expert in The Royal Court Affairs, Salalah) tested this mixture with ratio 1:1 (50% mustard oil and 50% liquid soap) at 0.5% v/v against whitefly adults, with promising results. The mixture was named by him; Naboil 50% (Personal communication, Abdel Salam). For this study the mixture ratio was changed to 3:1 mustard oil:soap because a 1:1 mixture showed phytotoxic effects on tomato leaves at higher rates (≥ 1%) whereas the 3:1 mixture did not. From the preliminary tests in chapter three, mustard oil 75% showed high mortality rates against SLW eggs when applied at 0.25% and against adults at 1%.

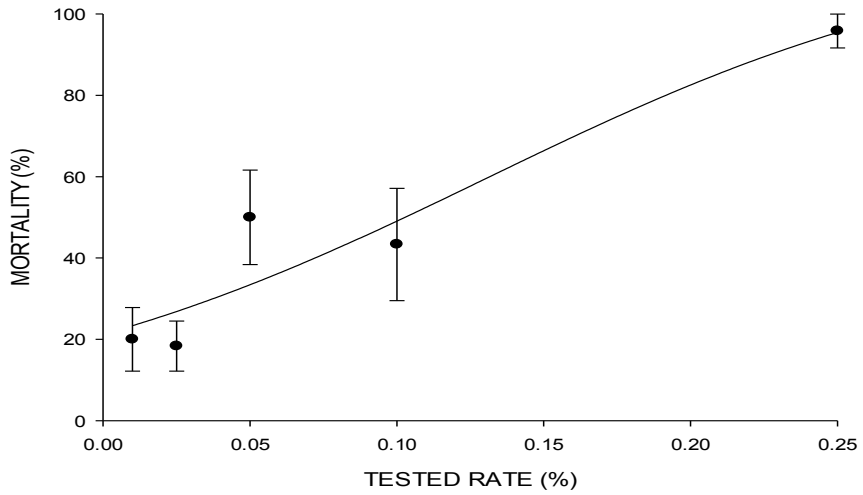


Figure 6.1: SLW egg mortality percentages (Mean ± SE) of mustard oil 75% and liquid soap at different tested rates.

When the formulation was tested on the egg stage, the mortality was high (95.8%) at 0.25% (Figure 6.1). Egg mortality at rates of 0.05% and 0.1% were 50% and 43.3%, respectively. Efficacy decreased at lower rates. For instance at 0.01% and 0.025%, the mortalities were 20% and 18.3%, respectively. The LD_{50} and LD_{90} values were estimated as 0.44% and 1.73%, respectively (Table 6.1). According to the analysis using probit analysis with 95% confidence interval, there was a significant difference between the different tested rates of mustard oil 75% against SLW eggs.

Table 6.1: Summary of toxicity of mustard oil 75% to the developmental stages of *B. tabaci* B biotype on tomato leaves in laboratory bioassays

Formulation	Stage	n	LD_{50}		LD_{90}		P
			(%) (± SE)	95% CI	(%) (± SE)	95% CI	
Mustard oil 75%	Egg	699	0.44 (± 0.028)	0.385 – 0.496	1.73 (± 0.188)	1.437 – 2.230)	<0.001
	Young nymph	635	0.13 (± 0.008)	0.118 – 0.150	0.44 (± 0.042)	0.368 – 0.542	<0.001
	Old nymph	549	0.55 (± 0.051)	0.468 – 0.676	2.91 (± 0.617)	2.041 – 4.806	<0.001
	Adult	549	0.42 (± 0.025)	0.375 – 0.474	1.06 (± 0.081)	0.929 – 1.261	<0.001

n: number of groups tested containing 30 individuals each,
LD_{50} and LD_{90} values are in %,
SE: Standard Error,
CI: Confidence Interval,
P: Significance of fitted model.

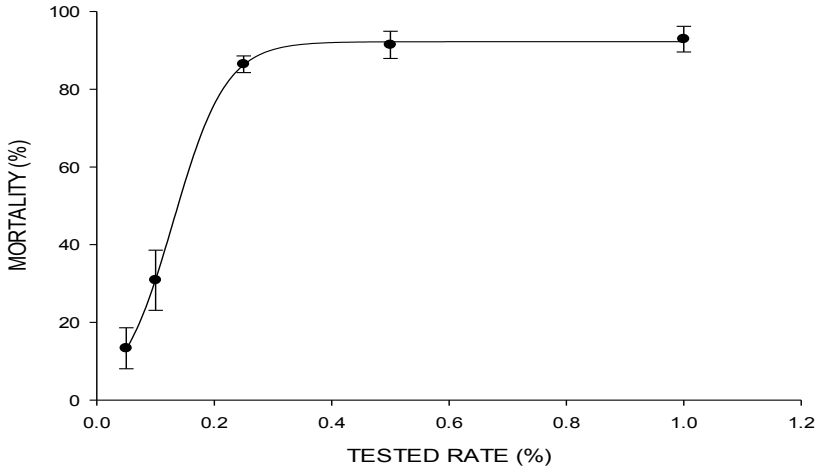


Figure 6.2: SLW young nymph mortality percentages (Mean ± SE) of mustard oil 75% and liquid soap at different tested rates.

Figure 6.2 presents the effects of mustard oil 75% against younger nymphs (first and second instars) of SLW. It showed that the lowest tested rates (0.05% and 0.1%) had low mortality (13.35% and 30.8%, respectively), but it was highly effective at the tested rate 0.25% and above (86.4%). The young nymphs were dried and discolored from pale yellow to dark brown.

The effectiveness of the mustard oil 75% against older nymphs (third and fourth instars) are given in figure 6.3. The low rates showed almost no effects on old nymphs. Higher rates (0.25%, 0.5% and 1%) resulted in mortalities of 47.4%, 34.9% and 65.2%, respectively. The dead older nymphs were flattened, dried and turned from yellow to brown in colour.

LD_{50} and LD_{90} values of both younger and older nymphs were calculated (Table 6.1). The LD_{50} values of younger and older nymphs were 0.13% and 0.55%, respectively whereas LD_{90} values were 0.44% and 2.91%, respectively. The results showed that younger nymphs were more sensitive to mustard oil than older ones. The reason could be the younger nymphs were delicate and their outer waxy layer was not well developed.

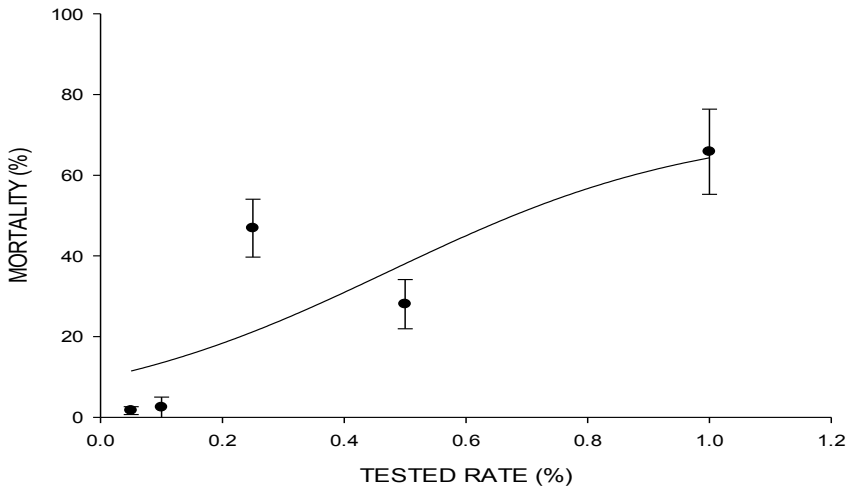


Figure 6.3: SLW old nymph mortality percentages (Mean ± SE) of mustard oil 75% and liquid soap at different tested rates.

From adult tests (Figure 6.4), at 0.25% and 0.5% mortalities were low; 34.0% and 37.0%, respectively. At 1% and above mortality was much higher: 94.17%, 95% and 100%, respectively at 1%, 1.5% and 2%. The LD_{50} and LD_{90} of the formulation against adults were 0.42% and 1.06%, respectively (Table 6.1).

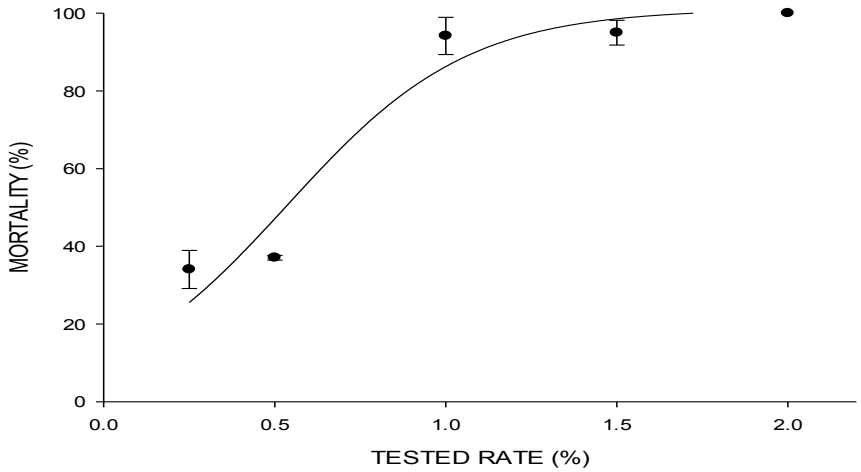


Figure 6.4: SLW adult mortality percentages (Mean ± SE) of mustard oil 75% and liquid soap at different tested rates.

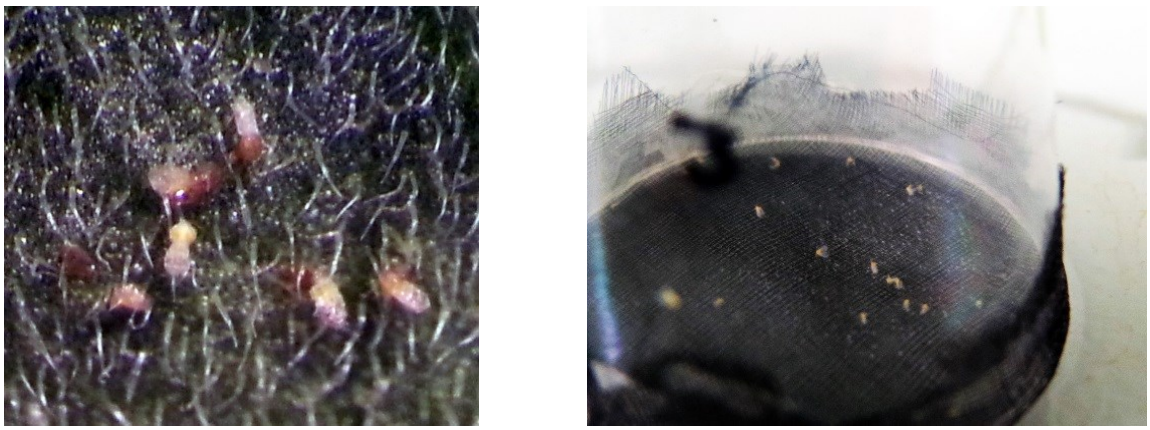


Figure 6.5: Dead nymphs partially emerged from egg shell after treatment with formulations (Left) and dead adults after exposure to a wet leaflet treated with mustard oil 75% (Right).

It is clear that the mixture of mustard oil 75% and liquid soap 25% showed lethal effects on all of the SLW developmental stages. A study by Puri et al. (1994) showed promising effects of detergents including Nirma® (Pate1 Detergents, Ahmedabad, India), Rin, Surf and Wheel (Hindustan Lever, Bombay, India) at concentrations of 0.5 and 1.0% and essential oils from cottonseed and neem were also effective. The mortality rates of nymphs and adults of SLW were 97 – 99% and 69 – 91%, respectively at 1% tested rate of the mixture. From our tests, the younger and older nymphs and adults were sensitive to the mixture of mustard oil and liquid soap. For example, at 1% the mortality rates were 93%, 65% and 94%, respectively. Another study by Yarahmadi et al. (2013) showed the contact toxicity of *Pelargonium roseum* Andrews (Geraniaceae) and *Artemisia sieberi* Besser (Asteraceae) essential oils on SLW. They found that the mortality rates of eggs and nymphs at 0.0012% were more than 95% after 48 h from treatment, whereas the rates 0.0125% and higher rate showed severe phytotoxic effect on cucumber leaves in glasshouse. In this study, the mortalities of lower rates (0.01% and 0.025%) of the mustard oil and soap were not effective against the SLW developmental stages but the mortalities of the eggs, younger nymphs and older nymphs reached 95% at 0.25%, 91% at 0.5% and 65% at 1%, respectively. Additionally, there were no phytotoxicity symptoms of the mixture (3:1) even at 5%. Recently, Iram et al. (2014) studied effect of some plant extracts of mint (*Mentha* spp.) (Lamiaceae) and geranium (*Pelargonium graveolens* L'Her) and soybean oil (*Glycine max* L. Merr) (Fabaceae), mustard oil (*Brassica* spp.) and taramera oil (*Eruca sativa* Miller) (Brassicaceae) against whitefly, *B. tabaci* on sesame crop. These extracts were mixed with castille soap as an emulsifier. The mustard oil was used at rate of 3% and sprayed 2 times at bi-weekly interval. They found that after 24 hours from applying the formulations, there were 34.8% and 88.7% reduction in the whitefly population in the two sprays, respectively, comparing with the whitefly population in control plants sprayed with water. A study

by Fazal (1998) also used mustard oil against whitefly and showed a reduction in whitefly population by 80 – 90%.

6.4. Conclusion

To conclude, in literature there were few previous studies of the effect of mustard oil against whitefly. Controlling insect eggs is often difficult because of the outer cover that protects them from outside environmental factors. However in this study, the formulation did kill eggs at 0.25%: observations included unhatched eggs, shriveled eggs and mortality of partially emerged nymphs (Figure 6.5, Left). Naranjo and Ellsworth (1999) recorded high levels of embryogenesis disruption in the eggs after spraying an insecticide. Their observations were consistent with the results of this study that the formulations have an ovicidal effect on the eggs of the silverleaf whitefly. Nymphal stages were also affected by the application of mustard oil mixed with liquid soap. Younger nymphs were more delicate and highly affected than older ones. These results agreed with previous studies by Puri et al. 1994 and Yang et al. 2010 studies when different essential oils tested against the nymphal stages of SLW. Additionally, mustard oil 75% showed insecticidal effects against adults at 1% and above (Figure 6.5, Right). Mustard oil could be used in botanical insecticidal formulations including surfactants and other essential oils to manage insect pests. Additionally, mustard oil formulations could have a promising role in integrated pest management programs in future.

CHAPTER 7: Evaluation of the Toxicity Effects of New Plant Essential Oil Formulations against the Developmental Stages of *Bemisia tabaci* B Biotype Under Laboratory and Glasshouse Conditions.

Abstract

In this study, mortality rates of three new plant essential oil formulations were tested against the developmental stages of SLW in the laboratory and the glasshouse. From the results, LD_{50} and LD_{90} values were estimated. The formulations were prepared as a result of several preliminary tests of surfactants and essential oils. These formulations were: formulation one (F1) containing mustard oil, MW-100 emulsifier, lauryl glucoside and cellosolve acetate (Diethylene glycol monomethyl ether); formulation three (F3) containing mustard oil, MW-100 emulsifier, laureth carboxylate and monoethanolamine; and formulation four (F4) containing mustard oil, MW-100 emulsifier, lauryl glucoside and monoethanolamine. These formulations were tested at different concentrations (0.25%, 0.44%. 0.69%, 1% and 1.23%) compared with the control (water) against egg and nymphal stages of SLW in the laboratory using a spraying method. The formulations were effective against egg and nymphal stages. At low tested rates (0.25% and 0.44%) the mortalities were less than 20% for eggs and nymphs. There was no effect of the formulations on adults at the above tested rates. However, when the rates were increased to 1.56%, 2.04%, 2.78%, 4% and 6.25%, there were phytotoxicity effects, and adult mortalities for F3 and F4 were less than 30% at all tested rates. F1 resulted in 38.54% mortality at the highest rate (6.25%). These formulations have an ovicidal effect of disrupting the embryogenesis process leading to egg death and also kill the nymphs. The formulations were also tested against the developmental stages of SLW under glasshouse conditions (0.25%, 0.44%. 0.69%, 1% and 1.23%).Whereas, these formulations had a low impact on adults under laboratory conditions they showed high mortalities under glasshouse conditions. The egg and nymphal stages, both younger and older nymphs, were less affected by the formulations under glasshouse trial comparing with the results under laboratory conditions. The results showed that the formulations could play an important role in managing SLW populations and could be a part of an IPM programs. Further studies will be needed to test their repellent and egg oviposition deterrent effects and also their impact on SLW natural enemies.

Key words: silverleaf whitefly (SLW), *Bemisia tabaci*, essential oil formulation, mortality rates.

7.1. Introduction

In general, conventional pesticides are most widely used in agricultural production. Use of conventional pesticides in agriculture has caused serious short and long term effects on environment and non-target species. Long term use of conventional pesticides caused resistance in insect pests. Use of botanical insecticides instead of chemical compounds is the cheaper, effective and alternative method for pest control (Regnault-Roger 1997; Isman 2000; Regnault-Roger et al. 2012; El-Wakeil, 2013).

Studies have been conducted on the insecticidal toxicity effect of certain plant essential oil formulations on all developmental stages of *B. tabaci*. Essential oil vapours from *Satureja hortensis* L., *Ocimum basilicum* L. and *Thymus vulgaris* L. (Lamiacae) were tested for their toxicities against the adults of *B. tabaci*. *Satureja hortensis* was found to be the most effective, compared with the other two species (Aslan et al. 2004). Ateyyat et al. (2009) tested nine plant extracts, which have medicinal activity, *Achillea biebersteinii* L. (Asterales: Asteraceae), *Artemisia inculta* Del. (Asterales: Asteraceae), *Ballota undulata* Benth. (Lamiales: Lamiaceae), *Euphorbia hierosolymitana* Boiss. (Malpighiales: Euphorbbiaceae), *Galium longifolium* (Sibth. and SM.) (Gentianales: Rubiaceae), *Lepidium sativum* L. (Brassicales: Brassicaceae), *Pimpinella anisum* L. (Apiales: Apiaceae), *Phlomis syriaca* Boiss. (Lamiales: Lamiaceae) and *Retama raetam* (Forssk.) Webb and Berthel (Fabales) against the developmental stages of *B. tabaci*. They found that the extracts of *L. sativum* killed 71 % of early of the stage nymphs. Yang et al. (2010) and Cruz-Estrada et al. (2013) also studied the toxicity of several essential oils against whitefly and found some of them had toxicity effects. From several preliminary tests, some surfactants and essential oils showed promising effects against the developmental stages of *B. tabaci* B biotype. Five formulations were prepared containing a mix of the most effective products and were tested in this experiment against the eggs, nymphs and adults of SLW. The objective of this experiment was to evaluate different plant essential oil formulations, containing surfactants and mustard oil, for efficacy against the developmental stages of silverleaf whitefly at different concentrations under laboratory and glasshouse conditions.

7.2. Materials and Methods

7.2.1. Whitefly and tomato seedlings

The methods for rearing whitefly and plant seedlings are described in chapter three section 3.2.1.

7.2.2. Essential oil formulations

The components of the five formulations and the percentage proportions of each one were as in table 7.1. MW 100 was used as an emulsifier (APCJ 2006). The phytotoxic effects of these formulations were tested to determine the proper tested rates.

Table 7.1: The components of the five formulations used in this experiment

Formulation 1	%
Lauryl glucoside	20
MW 100	40
Mustard oil	20
Cellosolve acetate	20

Formulation 2	%
Laureth carboxylate	20
MW 100	40
Mustard oil	20
Cellosolve acetate	20

Formulation 3	%
Laureth carboxylate	20
MW 100	40
Mustard oil	20
Monoethanolamine	20

Formulation 4	%
Lauryl glucoside	20
MW 100	40
Mustard oil	20
Monethanolamine	20

Formulation 5	%
Lauryl glucoside	20
MW 100	40
Neem oil	20
Cellosolve acetate	20

The five formulations were firstly tested to determine their impact on the tomato leaves. 1% v/v was used and phytotoxicity effects were very severe when F2 and F5 were sprayed on the leaves. F2 and F5 were then tested at a low concentration (0.44%) but still showed phytotoxicity symptoms. Therefore, F2 and F5 were omitted and F1, F3 and F4 were used to be tested for the toxicity effects against SLW developmental stages under laboratory and glasshouse conditions. Water was used as a control treatment.

7.2.3. Mortality test procedures under laboratory conditions

7.2.3.1. Tests against egg stage

The formulations were tested against three day old eggs at different rates (0.25%, 0.44%. 0.69%, 1%, and 1.23%) using the method procedure in chapter six section 6.2.3. Egg mortality percentage was calculated by counting unhatched eggs and shriveled eggs.

7.2.3.2. Tests against nymphal stages

For the nymphal stage tests, different rates of the formulations (0.25%, 0.44%. 0.69%, 1%, and 1.23%) were used to establish the efficacy according to the methodology described in chapter five section 5.2.3. The nymphs were categorized into younger nymphs including first and second nymphal instars and older ones including third and fourth instars.

7.2.3.3. Tests against adult stage

To evaluate the mortality rates of three formulations, the method procedure described in chapter four section 4.2.3 was used. Firstly, the rates of the formulations were: 0.25%, 0.44%, 0.69%, 1% and 1.23%. However, these rates did not show effects on adults then the rates were increased to 1.56%, 2.04%, 2.78%, 4% and 6.25%.

7.2.4. Mortality test procedure under glasshouse conditions

Based on the results of the laboratory tests three formulations (F1, F3 and F4) were selected for further testing against whitefly held under glasshouse conditions. The trial was conducted in a glasshouse in The University of Queensland, Gatton campus (T = 30 ± 2ºC and RH = 50% ± 10%) during January – February 2016. All the developmental stages of SLW were subjected to the three formulations (F1, F3 and F4) using a spraying method with a low pressure half liter hand-held sprayer. Four week old tomato seedlings were introduced into a glasshouse bay containing a whitefly colony. The seedlings were left to be infested with whitefly. After eight weeks (two generations), adults, eggs and young and old nymphs of the SLW were present on the lower surface of the tomato leaves. Half liter of the three formulations were applied at different rates, 0.25%, 0.44%, 0.69%, 1% and 1.23% as a spray to the leaves of the plants. Water was used as a control.

Two plants were assigned to each treatment. Before spraying, old leaves were removed from the tomato seedlings and five leaves (each leaf with seven or nine leaflets) were kept in each seedling. There were around 80 leaflets per treatment. The seedlings were sprayed until run-off. Ten replicates were used. Each replicate was a 3 cm diameter leaf disk sample. The leaf disks were selected from the same site of the leaflets for each immature stage of SLW. For example, for eggs, the leaf disks were selected from the middle of the leaflets because we observed that females prefer

to lay more eggs in that site, whereas from the top half of the leaflets for both young and old nymphs because the newly emerged nymphs crawl and mainly prefer to settle in that site.

Mortalities were estimated in the leaflets of the upper young leaves for eggs and in the middle leaves for the younger nymphs. Old nymph mortality rates were estimated from the terminal leaflets of the lower leaves. Numbers of adults, eggs and nymphs were counted 24 hours before spraying. Mortality rates were assessed 24 hours after spraying in the case of the adults and 10 days after spraying for eggs and nymphs.

Twenty four hours before spraying the tomato seedlings in the glasshouse bay, adult numbers were counted from 20 randomly selected leaflets by slowly turning the leaf and counting the adults on the lower surface. The average estimated adult number (EAN) was 20 adults per leaflet. The two plants in each treatment were covered from three directions before spraying and the covering sheet was held for a minute to reduce transferring the adults from sprayed plants to others in the glasshouse. Twenty four hours after spraying, adult mortalities were estimated by counting the live adults on 10 randomly selected leaflets for each treatment. Then, the means were calculated to be compared with the EAN. Mortality rates then were calculated. The number of adults in the control treatment (water) was more than the EAN. In this case, corrected efficacy percentages of the formulations (F1, F3 and F4) were calculated using Sun – Shepard's formula (section 7.2.5 statistical analysis). The estimated numbers of eggs, young and old nymphs were 100, 120 and 80, respectively. These numbers were calculated from 20 randomly selected 3 cm diameter leaf disks from the control treatment. The toxicity effects on the SLW developmental stages used in chapter seven were used here to estimate the mortalities.

7.2.5. Statistical analysis

Mortality data from all tested concentrations of formulations were analyzed using Minitab 17. Probit analysis was used to estimate LD_{50} and LD_{90}. Regression analysis was used to determine relationships between percentages of mortality and tested rates of the formulations. Corrected efficacy percentages of the formulations under the glasshouse conditions were calculated using Sun – Shepard's formula (Püntener 1981).

$$\text{Corrected \%} = \left(\frac{\text{Mortality \% in treatment} \pm \text{Change \% in control}}{100 \pm \text{Change \% in control}} \right) * 100$$

Data were subjected to General Linear Model ANOVA and Tukey's test to identify treatments and tested rates that had significant differences between them. Results were assessed at the 95% confidence level. Mortality rates with standard errors were presented using the Sigma Plot program.

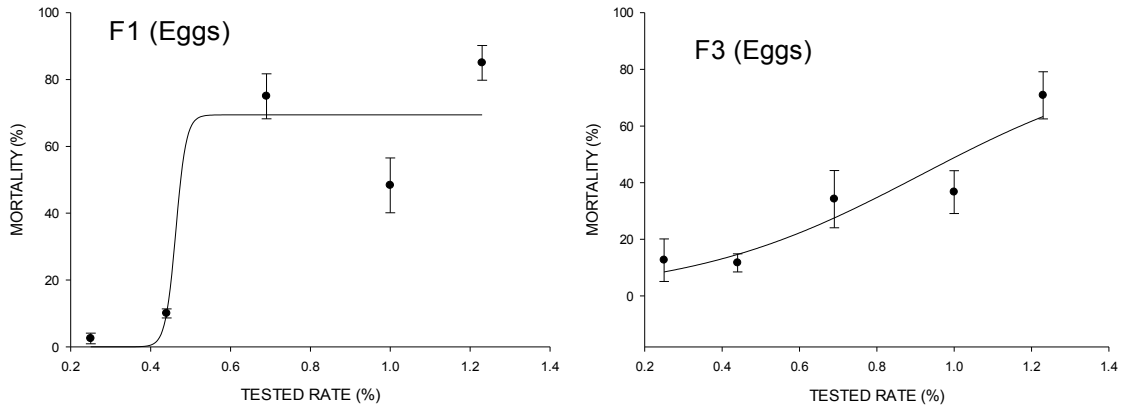
7.3. Results and Discussion

7.3.1. Toxicity effect of formulations under laboratory conditions

7.3.1.1. Toxicity effect of formulations against SLW eggs

Figure 7.1 presents the results of the three formulations; F1, F3 and F4, against silverleaf whitefly eggs (three day old). All formulations showed different mortality rates at each tested rate. At a concentration of 1.23%, mortality of the F1, F3 and F4 formulations were 85%, 70.8% and 69.2%, respectively. Therefore, the tested rates were reduced to 0.25%, 0.44%, 0.69% and 1% to estimate the LD_{50} and LD_{90} of the formulations. There was no big difference in mortalities of the formulations F3 and F4 against eggs when tested at all rates. However, the mortality rates of F1 were higher than F3 and F4 at tested rates of 0.69%, 1% and 1.23%. It showed higher mortality rate at 1%, which comprised 67.5%.

Lethal doses (LD_{50} and LD_{90}) of the tested formulations (F1, F3 and F4) against the eggs of SLW are presented in table 7.2. The LD_{50} value of F1 was the lowest (0.73 %) and F3 and F4 had the highest LD_{50} values, 1.02 and 1.05%, respectively. Simultaneously F3 and F4 had the highest LD_{90} values, 3.43 and 2.69%, respectively whereas the LD_{90} of F1 was 1.59%.



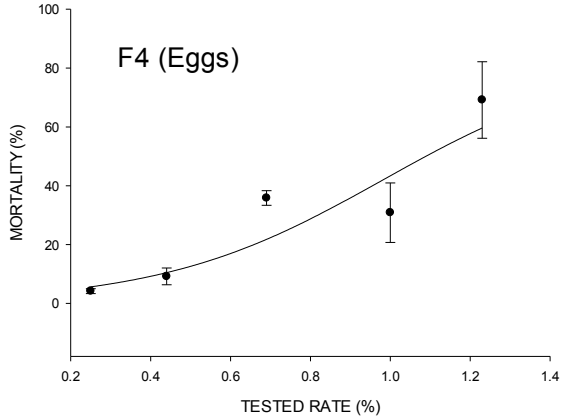
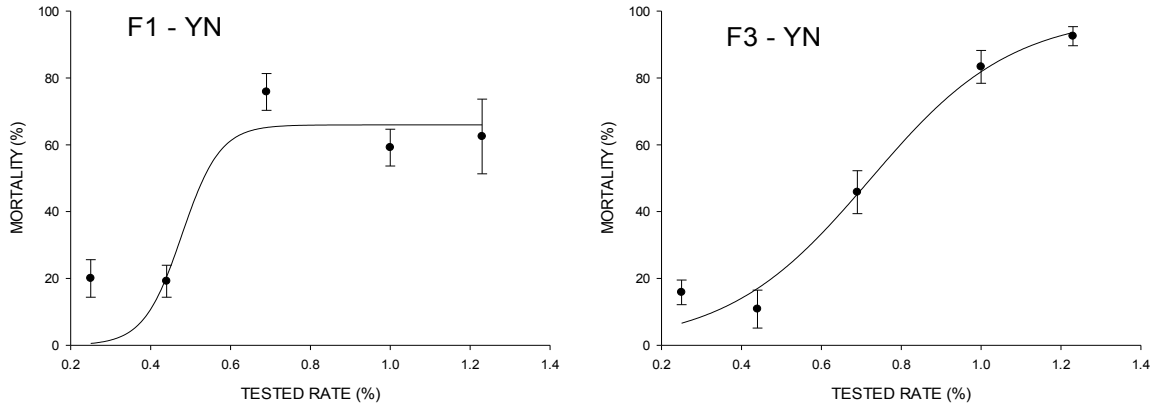


Figure 7.1: Mortality rates of three formulations against SLW eggs at different concentrations

7.3.1.2. Toxicity effect of formulations against SLW nymphs

The graphs in figure 7.2 presented the means ± SE results of the toxicity of the three formulations against young nymphs, first and second instars. At low rates, 0.25% and 0.44%, mortality rates of all formulations were low; less than 20%. At 0.69%, F1 showed the highest mortality rate. It was 75.83% whereas F3 and F4 mortalities of 45.83% and 14.17%, respectively. When the tested rates of the formulations were increased to 1% and 1.23%, mortality rates were also increased. Mortalities of formulations (F1, F3 and F4) were (59.17% and 62.5%), (83.33% and 92.5%) and (68.33% and 80%), respectively.

The LD_{50} and LD_{90} values of the formulations on the young nymphs were estimated (Table 7.2). There was no difference between LD_{50} values of F1 and F3. They were 0.69% and 0.65%, respectively. However, the LD_{50} value of F4 was the highest among them, 0.88%. F1 had the highest LD_{90} value (3.15%) whereas the F3 and F4 had values of 1.36% and 1.68%, respectively.



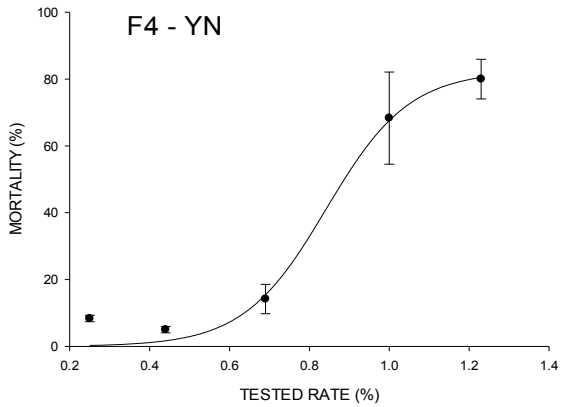


Figure 7.2: Mortality rates of three formulations against SLW younger nymphs (1st and 2nd instars) at different concentrations.

Figure 7.3 shows the results of the effect of the three new formulations (F1, F3 and F4) on older nymphs, third and fourth instars. Mortalities of older nymphs were very low at the lower tested rates; 0.25% and 0.44%. They were less than 20% for all three formulations. At 0.69%, there were no differences in mortality rates between F1 and F3. There were 45% and 45.83%, respectively, whereas F4 was less effective (35.83%). Older nymphs were more affected by the formulations (F1, F3 and F4) at 1.23% and the mortality rates were 62.5%, 88.33% and 71.67%, respectively.

The values of the LD_{50} and LD_{90} of the three formulations against old nymphs were calculated (Table 7.2). There were no differences between the LD_{50} values of the formulations. They were 1.03%, 0.91% and 0.9%, respectively. However, the difference was clear in the LD_{90} values of the formulations. F1 had the highest value (2.53%) whereas F3 and F4 values were 1.81% and 2.4%, respectively.

From figures 7.2 and 7.3, both younger and older nymphs were little affected by the formulations at low tested rates, 0.25% and 0.44%. When formulations were applied at a rate of 1.23%, young and old nymphs were similarly affected. However, younger nymphs were more sensitive to F3 and F4 than older ones. Our hypothesis from using surfactants was to remove and/or disrupt the outer wax layer of the nymphal stages of SLW. Results showed that this did occur, and showed an excellent impact. Additionally, the formulations give the same results as the surfactant alone. These formulations have insecticidal effects on the nymphal stage of SLW.

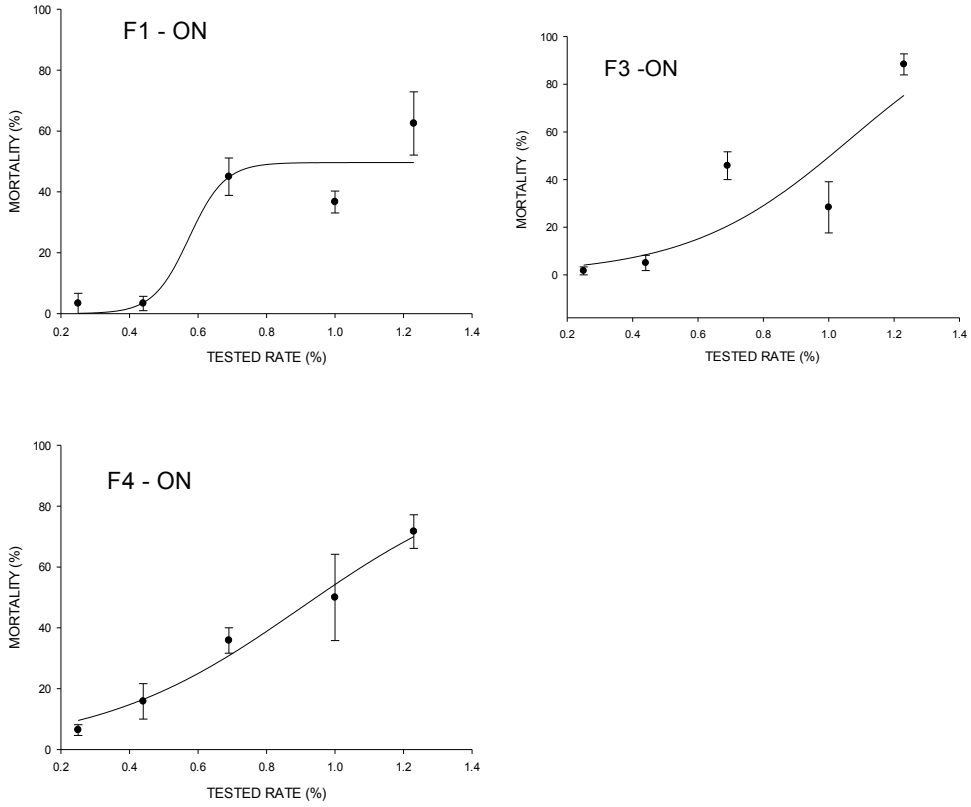


Figure 7.3: Mortality rates of three formulations against SLW older nymphs (3rd and 4th instars) at different concentrations.

7.3.1.3. Toxicity effect of formulations against SLW adults

There were no effects of the formulations when they were applied against adults at the rates used against egg and nymphal stages (0.25%, 0.44%, 0.69%, 1% and 1.23%). Figure 7.4 presents the results of mortality rates of the formulations at higher rates (1.56%, 2.04%, 2.78%, 4% and 6.25%). Although the phytotoxicity effect was severe, the adult mortalities were low at all tested rates. The mortality was less than 30% except for F1 at 6.25% which showed a 38.54% adult mortality. Therefore, the LD_{50} and LD_{90} could not be calculated. These results were unexpected especially as one component of the formulation was monoethanolamine (MEA) that showed high mortality rates when sprayed alone. In this case, maybe there was no synergistic effect of MEA when it was added to the formulations. Another reason was that the adults were not subjected to the formulations directly but the leaflets were sprayed first and then the adults introduced. The adults could stay away from the wet leaflet surface for some time until it dried.

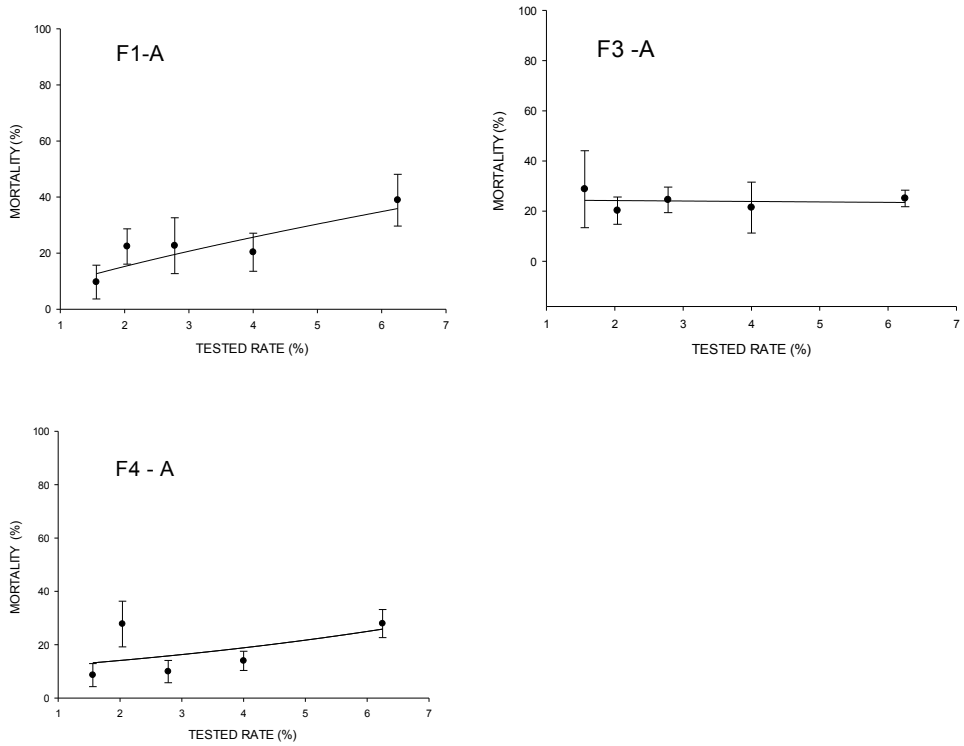


Figure 7.4: Mortality rates of three formulations against SLW adults at different concentrations.

Table 7.2: The LD_{50} and LD_{90} values of three formulations against the developmental stages of SLW under laboratory conditions:

Formulations	SLW Stage	n	LD_{50}		LD_{90}		P
			(%) (± SE)	95% CI	(%)(± SE)	95% CI	
F1	Egg	600	0.73 (± 0.026)	0.684 – 0.789	1.59 (± 0.117)	1.405 – 1.885	<0.001
F3	Egg	599	1.02 (± 0.062)	0.913 – 1.169	3.43 (± 0.601)	2.580 – 5.299	<0.001
F4	Egg	600	1.05 (± 0.052)	0.958 – 1.168	2.69 (± 0.348)	2.172 – 3.685	<0.001
F1	YN	600	0.69 (± 0.042)	0.608 – 0.778	3.15 (± 0.60)	2.317 – 5.061	<0.001
F3	YN	600	0.65 (± 0.023)	0.608 – 0.698	1.36 (± 0.086)	1.213 – 1.565	<0.001
F4	YN	600	0.88 (± 0.028)	0.830 – 0.942	1.68 (± 0.114)	1.500 – 1.971	<0.001
F1	ON	600	1.03 (± 0.048)	0.944 – 1.139	2.53 (± 0.305)	2.076 – 3.390	<0.001
F3	ON	600	0.91 (± 0.031)	0.854 – 0.976	1.81 (± 0.136)	1.589 – 2.152	<0.001
F4	ON	589	0.90 (± 0.041)	0.829 – 0.994	2.40 (± 0.285)	1.968 – 3.187	<0.001

n: number of groups tested containing 30 individuals each,
LD_{50} and LD_{90} values are in %,
SE: Standard Error,
CI: Confidence Interval,
P: Significance of fitted model.

7.3.2. Toxicity effect of formulations under glasshouse conditions

7.3.2.1. Toxicity effect of formulations against SLW adults

When the tomato seedlings were sprayed with the three formulations in the glasshouse bay, the SLW adults were directly subjected to the formulations diluted into different rates including 0.25%, 0.44%, .69%, 1% and 1.23%. From the graphs (Figure 7.5), it was clear that the formulations were effective against SLW adults. Generally, there were no differences between tested rates in effectiveness. The average mortality rates of the formulations (F1, F3 and F4) were 71.8%, 73.1% and 86.2%, respectively. The adults died instantly after spraying.

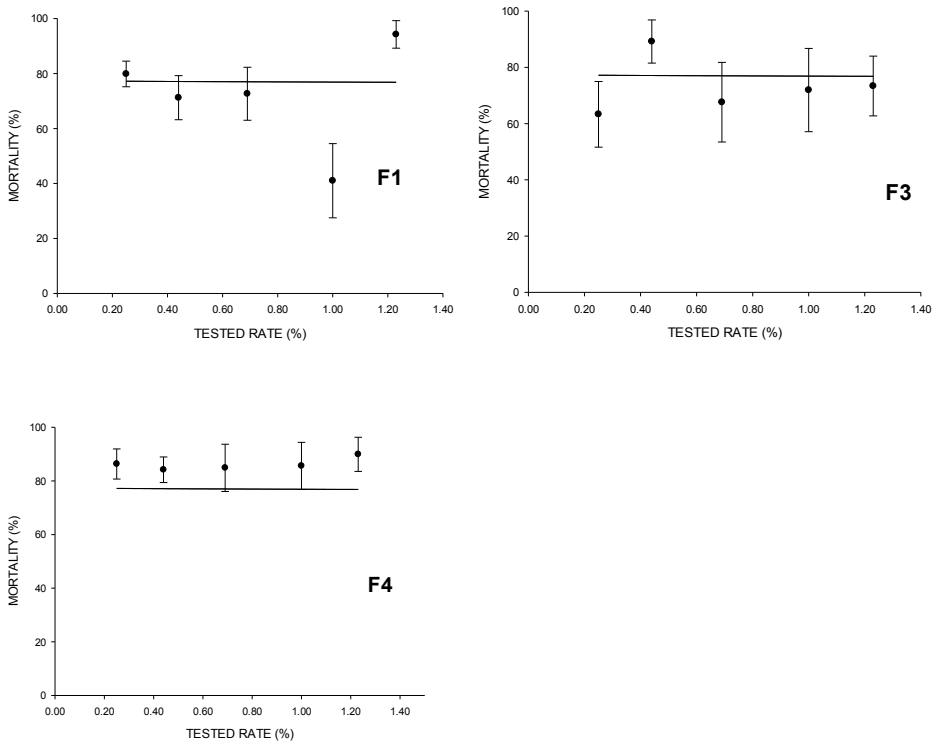


Figure 7.5: Mortality rates of three formulations against SLW adults at different concentrations under glasshouse conditions.

7.3.2.2. Toxicity effect of formulations against SLW eggs

From this glasshouse trial, figure 7.6 shows the results of the three formulations; F1, F3 and F4, against SLW eggs. All formulations showed different mortality rates at different tested rates. At the highest used concentration (1.23%), mortality of the F1, F3 and F4 formulations were 44.1%, 69.8% and 74.3%, respectively. The data were used to estimate the LD_{50} and LD_{90} of the formulations. There was no big difference in mortalities of the formulations F3 and F4 against eggs when tested at all rates. However, the mortality rates of F1 were lower than F3 and F4 at all tested rates. F3 and F4 showed higher mortality rate at 1%, which comprised 62.6% and 63.4%, respectively.

Lethal doses (LD_{50} and LD_{90}) of the tested formulations (F1, F3 and F4) against the eggs of SLW are presented in table 7.3. The LD_{50} value of F1 was the highest (1.25 %). On the other hand, F3 and F4 had the lowest LD_{50} values, 0.83% and 0.66%, respectively. At the same time F3 had the lowest LD_{90} values, 1.9% whereas the LD_{90} of F1 and F4 were 2.29% and 2.13%, respectively.

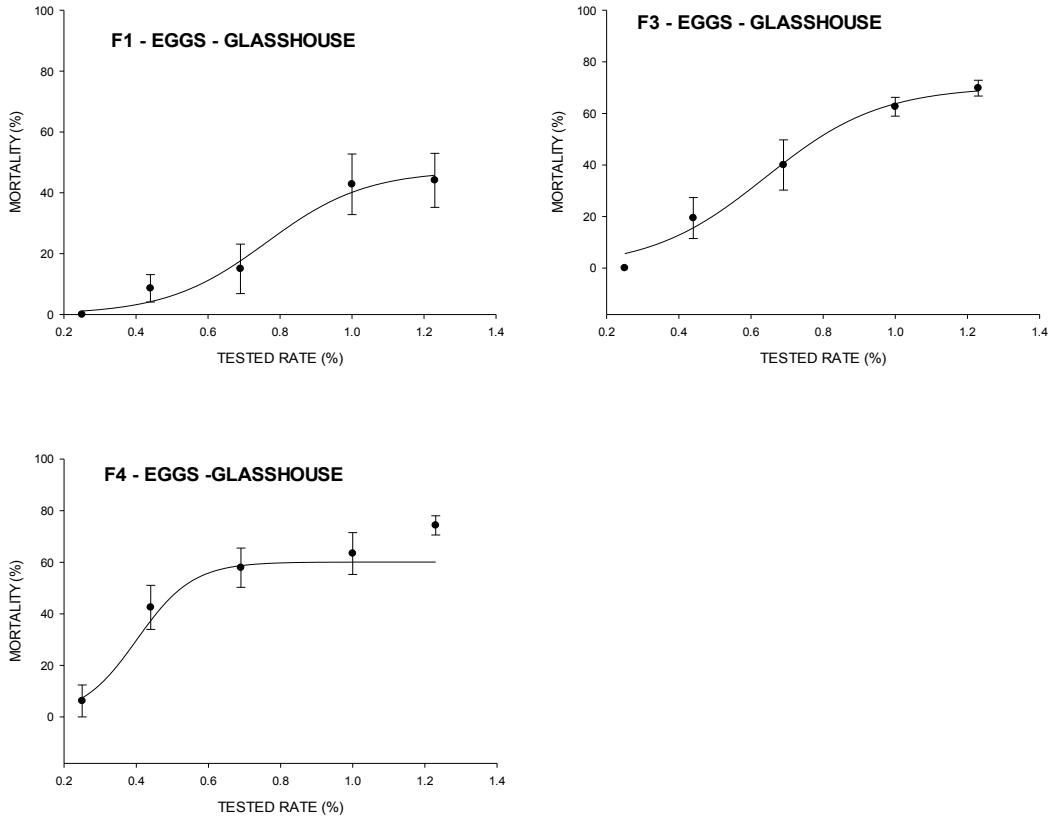


Figure 7.6: Mortality rates of three formulations against SLW eggs at different concentrations under glasshouse conditions.

7.3.2.3. Toxicity effect of formulations against SLW nymphs

When tested under laboratory conditions, the younger nymphs were sensitive to the formulations. The younger nymphs were then subjected to the same formulations under glasshouse conditions. The graphs in figure 7.7 presented the means ± SE results of the toxicity of the three formulations against younger nymphs. At low rates, less than 1%, mortality rates of all formulations were low against younger nymphs. At 1%, F3 showed the highest mortality rate among the two formulations, F1 and F4. It was 58.75% whereas F1 and F4 mortalities were similar. They were 45.67% and 45.08%, respectively. When the tested rates of the formulations were increased to 1.23%, mortality

rates were also increased to 78.5% for F1 and to 64.75% for F3 whereas the mortalities of F4 decreased to 39.75%.

The LD_{50} and LD_{90} values of the formulations on the young nymphs were estimated (Table 7.3). There were no differences between LD_{50} values of F1 and F3. They were 0.96% and 0.75%, respectively. However, the LD_{50} value of F4 was the highest among them, 2.85%. F1 had the lowest LD_{90} value (1.87%).

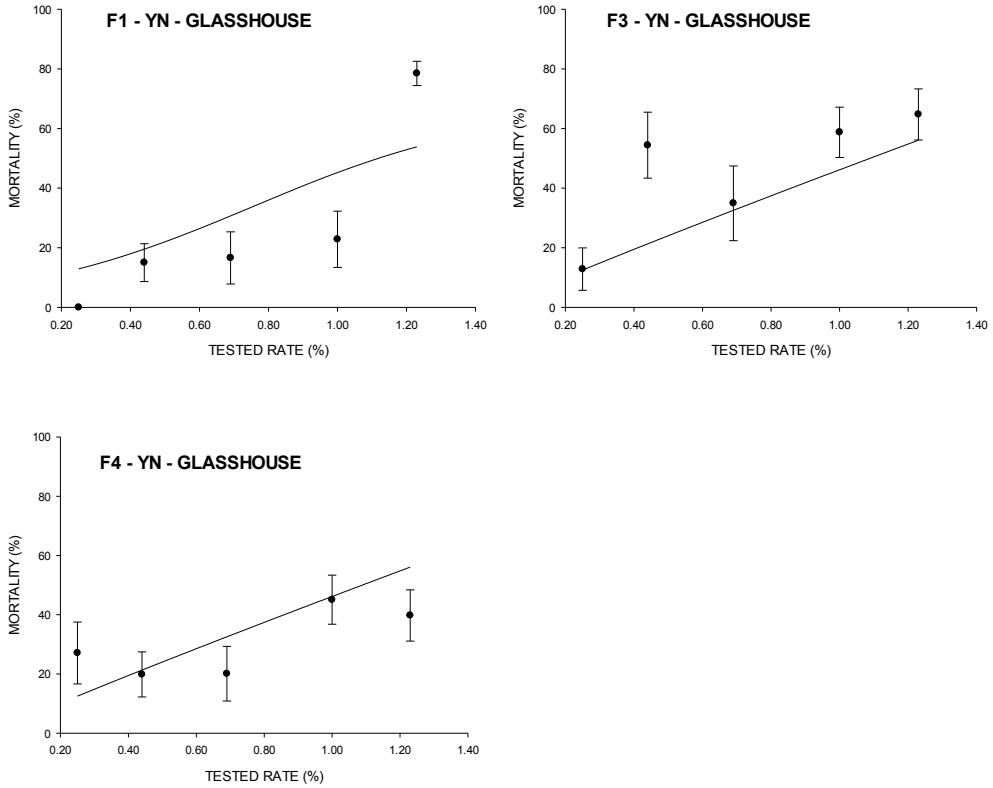


Figure 7.7: Mortality rates of three formulations against SLW young nymphs at different concentrations under glasshouse conditions.

The new formulations were also tested against older nymphs under glasshouse conditions. Figure 7.8 presents the results of the effect of the three formulations (F1, F3 and F4) on older nymphs. Mortalities of older nymphs were low at the lower tested rates; 0.25%, 0.44% and 0.69%, with an exception for F4 at 0.69% that showed mortality rate of 51.38%. At 1%, mortalities of F1 were the lowest (31.38%). and the mortalities of F3 and F4 were less than 50%. Older nymphs were more affected by the formulations of F1, F3 and F4 at 1.23% and the mortality rates were 67.5%, 60.75% and 59.6%, respectively.

The values of the LD_{50} and LD_{90} of the three formulations against old nymphs were calculated (Table 7.3). There were no significant differences between the LD_{50} values of F3 and F4. They were 1.00% and 0.95%, respectively. However, the difference was clear in the LD_{90} values of the formulations. F1 had the highest value (13.37%) whereas F3 and F4 values were 6.10% and 4.48%, respectively.

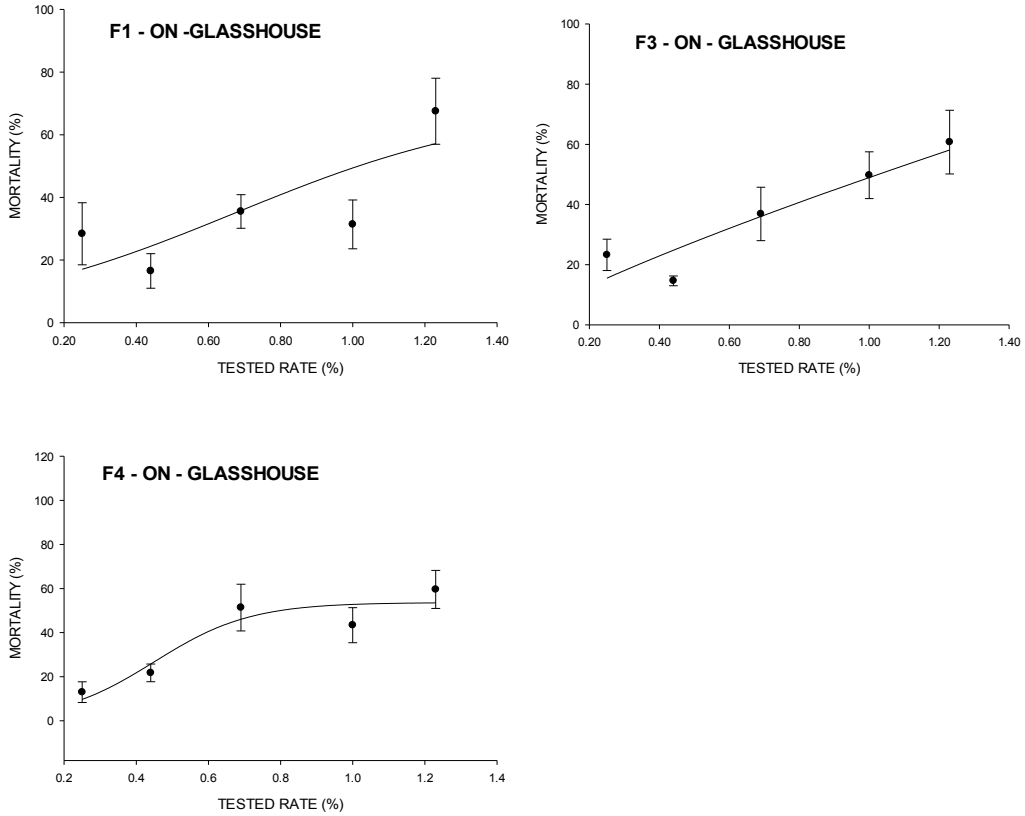


Figure 7.8: Mortality rates of three formulations against SLW old nymphs at different concentrations under glasshouse conditions.

Table 7.3: The LD_{50} and LD_{90} values of three formulations against the developmental stages of SLW under glasshouse conditions:

Formulations	SLW Stage	n	LD_{50}		LD_{90}		P
			(%)(± SE)	95% CI	(%)(± SE)	95% CI	
F1	Egg	500	1.25 (± 0.077)	1.122 – 1.447	2.29 (± 0.458)	2.280 – 4.379	<0.001
F3	Egg	500	0.83 (± 0.035)	0.768 – 0.907	1.90 (± 0.181)	1.616 – 2.380	<0.001
F4	Egg	500	0.66 (± 0.035)	0.588 – 0.730	2.13 (± 0.286)	1.705 – 2.950	<0.001
F1	YN	600	0.96 (± 0.032)	0.901 – 1.031	1.87 (± 0.144)	1.639 – 2.239	<0.001
F3	YN	600	0.75 (± 0.055)	0.652 – 0.879	4.58 (± 1.218)	3.020 – 9.193	<0.001
F4	YN	600	2.85 (± 1.292)	1.59 – 21.073	150.10 (± 238.575)	20.583 – 215265	<0.001
F1	ON	400	1.232 (± 0.212)	0.944 – 2.078	13.37 (± 8.726)	5.408 – 121.246	<0.001
F3	ON	400	1.00 (± 0.108)	0.833 – 1.318	6.10 (± 2.312)	3.474 – 13.398	<0.001
F4	ON	400	0.95 (± 0.084)	0.808 – 1.17	4.48 (± 1.300)	2.874 – 9.980	<0.001

n: number of groups tested containing 30 individuals each,
LD_{50} and LD_{90} values are in %,
SE: Standard Error,
CI: Confidence Interval,
P: Significance of fitted model.

7.3.2.4. A comparison between the toxicity effect of formulations against SLW developmental stages under laboratory and glasshouse conditions

Due to adverse phytotoxicity effects of the formulations on the tomato leaves at 1.23% in both laboratory and glasshouse trials, 1% was considered as the highest rate that could be used for these formulations. Accordingly, a comparison between the toxicity effects of the three formulations (F1, F3 and F4) at 1% under lab and glasshouse conditions is presented in figure 7.9.

Adult mortality rates were less than 20% in the lab. However, under glasshouse conditions the adults were more sensitive to the formulations (F1, F3 and F4). The mortalities increased to 41%, 71.9% and 85.6%, respectively. This difference could be due to the spraying method. In the laboratory trial, the leaflets were first sprayed then, the adults inside the clip cages, introduced immediately while the leaves were still wet. The adults were observed to move away from the leaflet for some time. In this case, the adults were not subjected to the formulations directly as in the glasshouse trial.

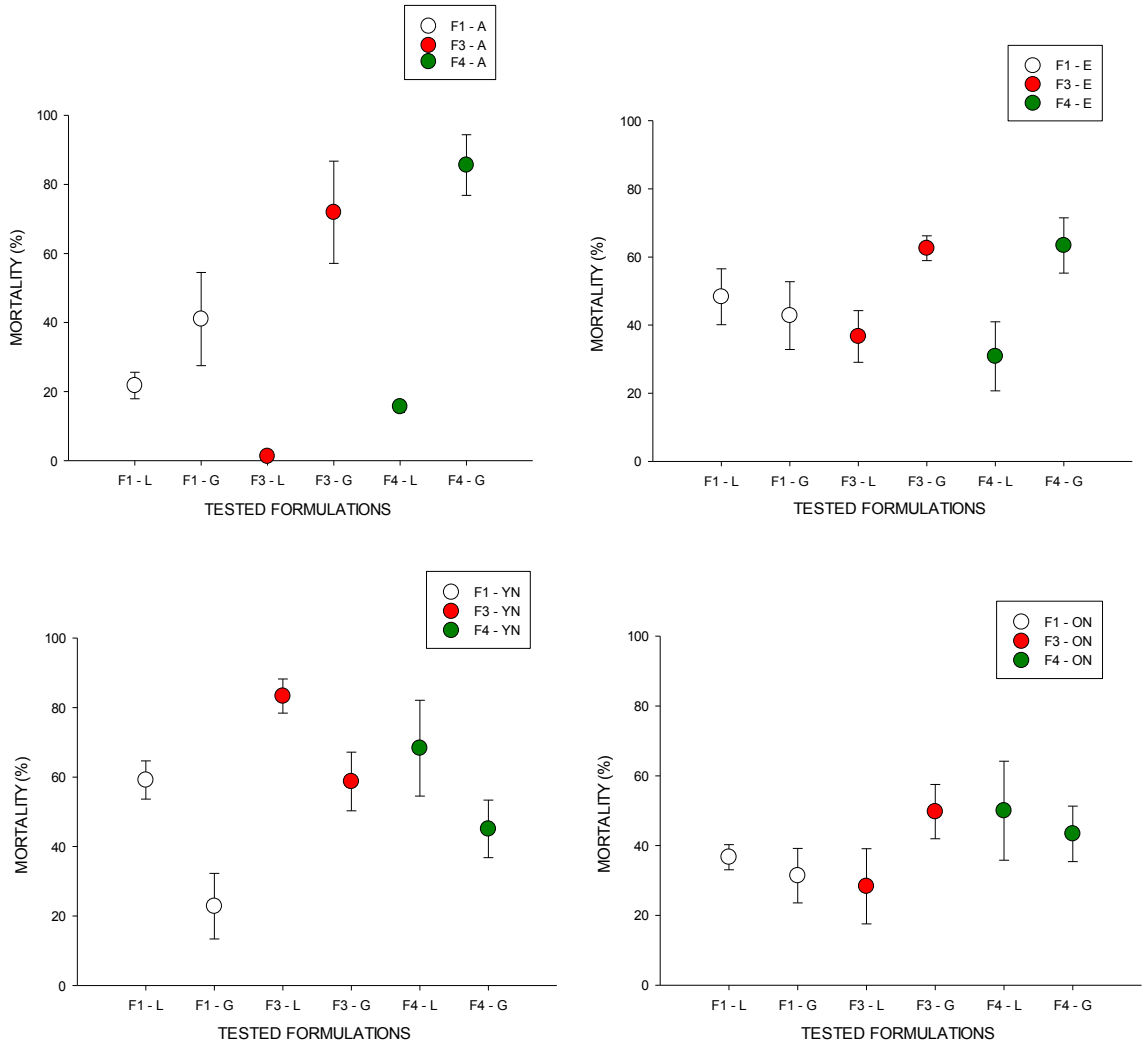


Figure 7.9: Mortality rates of three formulations against SLW developmental stages (A: Adult, E: Egg, YN: Young nymph and ON: Old nymph) at 1% under laboratory (L) and glasshouse (G) conditions.

In general, the effectiveness of the formulations against SLW nymphs were reduced in the glasshouse compared to the laboratory. The mortality rates of F1, F3 and F4 against younger nymphs at 1%, were 59.2%, 83.3% and 68.3%, respectively in the laboratory. However, the mortalities were reduced to 22.8%, 58.8% and 45.1%, respectively under glasshouse conditions. Under laboratory conditions, older nymphs were the least affected of the developmental stages of SLW. The mortalities were less than 50% of all formulations. The mortality rates of the formulations F1 and F4 against older nymphs also showed a reduction in the glasshouse compared to the laboratory but F3 showed a slight increase from 43.4% to 50%. This could be due to the spraying coverage. In the laboratory, the leaves were completely sprayed and the solutions contacted with the nymphs. While in the glasshouse, there were some difficulties to cover whole

leaves with the sprayed solutions due to the presence of most of the nymphs in close to the leaflet tips on the lower side of the leaflets and some leaves were cupped down.

The differences between the mortalities of egg stage in both laboratory and glasshouse conditions are presented in the figure 7.9. F1 showed a slight decrease in mortalities from 48.3% in the laboratory to 42.8% in the glasshouse. However, when F3 and F4 were sprayed in the glasshouse, the mortalities were doubled compared to the laboratory results. Most of the eggs were laid by SLW females in the middle of the leaflets and that made them more exposed to the sprayed solutions.

From the above results, it can be recognized that the three formulations were effective against all of the SLW developmental stages however with different mortality rates under laboratory conditions. For example in the laboratory, at the highest tested rate (1.23%) the mortality rates of the three formulations (F1, F3 and F4) against eggs were (85%, 70.8% and 69.2%, respectively). Younger nymph's mortalities (62.5%, 92.5% and 80%, respectively) were generally more affected by the formulations than the older ones (85%, 70.8% and 69.2%, respectively). However, the adults were less affected by the formulations. The mortality rate at 1.23% was less than 30%. From the literature, Al-mazra'awi and Ateyyat (2009) evaluated nine plant extracts against SLW adults and they found that not all the extracts affected the adults. Nine essential oil formulations were tested by Kim et al. (2011) against the B-biotype females using a spray bioassay. At 0.5%, formulations containing either garlic, cinnamon bark or vetiver Haiti oil resulted in 100% mortality. Whereas, oregano, catnip, clove leaf, davana and clove bud oils showed > 90% mortality. Garlic applied as 0.1% spray provided 100% mortality, whereas the toxicity of the other eight essential oil sprays was significantly lower. In another study by Baldin et al. (2015) the results showed 100% mortality of SLW adults when they exposed to an essential oil extracted from *Pelargonium graveolens* L'Her (Geraniaceae) at 0.5 μL L^{-1} in air. Similar results were observed by Çalmaşur et al. (2006) after essential oil vapours from *Micromeria fruticosa* L., *Nepeta racemosa* L. and *Origanum vulgare* L. (Lamiaceae) were tested for toxicities against the adults of SLW at a dose of 2 μl/l air and at 120 h of exposure.

Several studies evaluated essential oils against the immature stages of SLW, eggs and nymphs. Al-mazra'awi and Ateyyat (2009) found in their study that the percentage of unhatched eggs treated with nine aqueous plant extracts ranged between 0 and 33%. However, for the second nymphal instar, the extracts of *P. harmala*, *A. palaestina* and *R. chalepensis* resulted in 80, 77 and 67% mortality, respectively. For the third nymphal instar, *R. chalepensi*s and *A. strigosa* resulted in more than 50% mortality. Yang et al. (2010) observed that the essential oil extracted from garden thyme, *Thymus vulgaris* L. at 0.5% reduced the survival rate of *B. tabaci* by 73.4%, 79.0% and 58.2% after treatment of eggs, first nymphal and fourth nymphal instars, respectively,

Recently, Cruz-Estrada et al. (2013) studied six plant extracts in forms of ethanolic and aqueous extracts of *Acalypha gaumeri* L. (Euphorbiaceae), *Annona squamosal* L. (Annonaceae), *Carlowrightia myriantha* A. Gray (Acanthaceae), *Petiveria alliacea* L. (Phytolaccaceae) and *Trichilia arborea* C.DC. (Meliaceae). At the tested rate of 10 mg/ml, the ethanolic extracts caused high mortality rates (95 to 100%) on the eggs. Also, aqueous extracts caused mortalities ranging from 98 to 100% at tested rate of 3% w/v. Ethanolic extracts of all plants tested at the rate of 10 mg/mL caused high mortality (99-100%) on the nymphal stage. However, there were no insecticidal effects on *B. tabaci* nymphs were observed, with the exception of that of *C. myriantha*. When Kumar et al. (2005) tested the commercial neem, NeemAzal T/S 1% azadirachtin, at 10 ml/l, egg hatching was reduced to 51% and mortalities of the nymphs reached 100%. Generally, in comparison with previous studies, these results were similar to some extent to those reported with the effectiveness of essential oil formulations against the immature stages of SLW. However, for the toxicity of the formulations against adults, the adults have not been affected with some formulations (Cruz-Estrada et al. 2013) whereas they were very sensitive against others especially when the essential oil formulations were used as fumigants.

7.4. Conclusion

The plant essential oil formulations were tested in this study for the first time against one of the main agricultural pests, the silverleaf whitefly (SLW). The formulations (F1, F3 and F4) had an effective impact on the eggs of SLW, disrupting the embryogenesis process of the eggs. These formulations also affected all nymphal instars and showed mortality symptoms like dryness and shriveling. The adult stage was not affected severely in the laboratory trial. However, under glasshouse conditions, all three formulations showed promising adult mortality. Figure 7.10 showed the effects of the formulations on the SLW developmental stages. Furthermore, these new formulations have shown the possibility to be a part of SLW controlling measures and might play an important role in integrated pest management programs of SLW. Further experiments to determine the repellence and oviposition egg deterrent indices were conducted and presented in chapter eight.

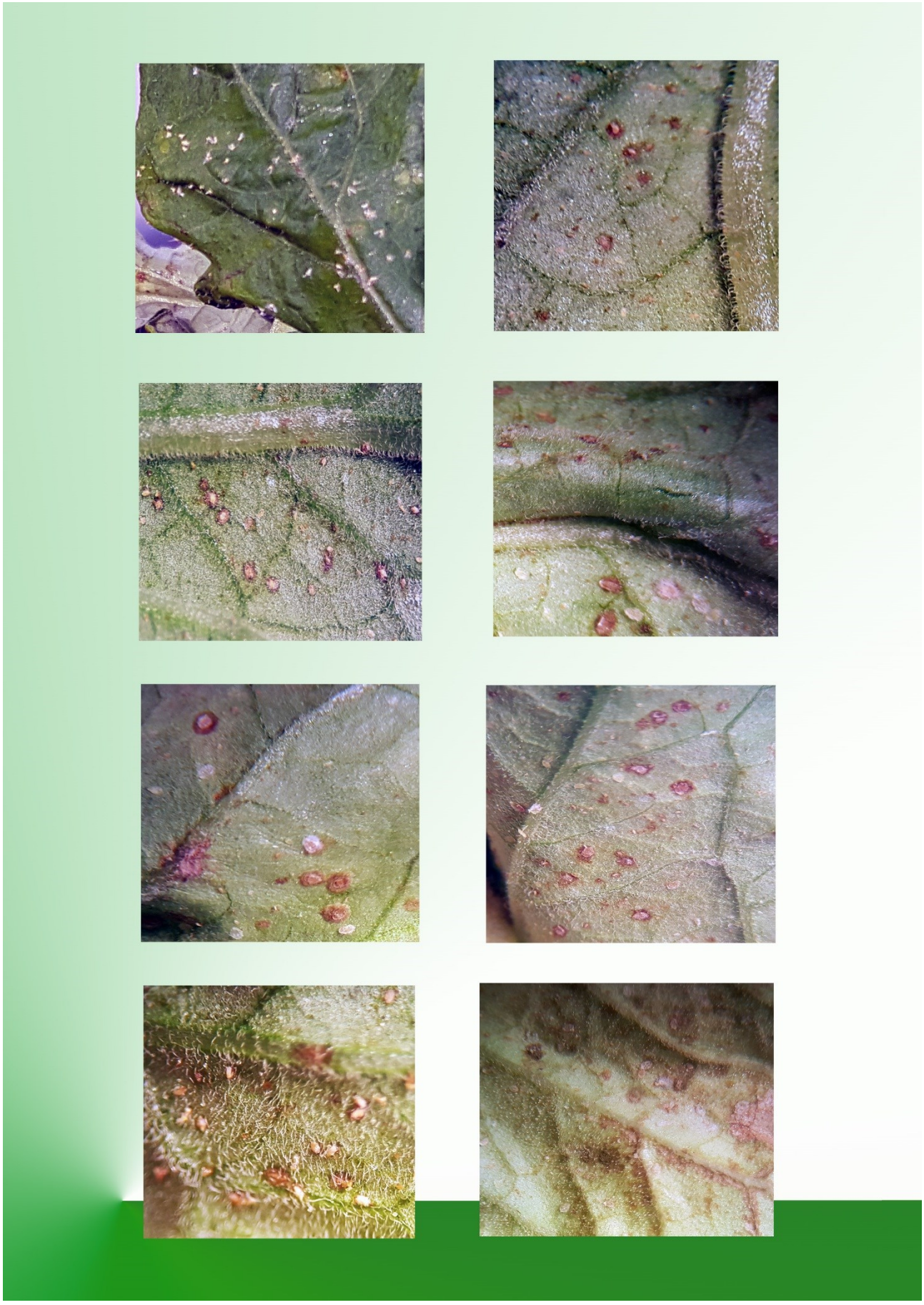


Figure 7.10: The effects of the three formulations on the developmental stages of SLW (eggs, young and old nymphs and adults).

CHAPTER 8: Repellence and Oviposition Deterrence Effects of New Plant Essential Oil Formulations against Adults of *Bemisia tabaci* B Biotype.

Abstract

Promising results of three new plant essential oil formulations against the silverleaf whitefly (SLW), *Bemisia tabaci* B biotype, were recorded in previous studies in this thesis. They showed insecticidal effects on the developmental stages of SLW. These products were: formulation one (F1) containing mustard oil, MW-100 emulsifier, lauryl glucoside (LG) and cellosolve acetate (DEGME), formulation three (F3) containing mustard oil, MW-100 emulsifier, laureth carboxylate (LEOCS) and monoethanolamine (MEA) and formulation four (F4) containing mustard oil, MW-100 emulsifier, LG and MEA. More biological studies were required such as determining their repellence and oviposition deterrence effects on SLW adults. In these experiments, the formulations were tested at 1.25%. Water was used as a negative control and neem oil as a positive control. The objective of this study was to determine the repellence index (RI) and oviposition deterrent index (ODI) of these new formulations. Choice and no-choice repellent tests under laboratory conditions were used in this study. Numbers of attracted adults were counted after 2, 6, 12, 24 and 48 h of the adult introduction. After 48 h, the mean numbers of laid eggs were calculated. Data were subjected to two-way ANOVA with Tukey's test. From the results of choice tests, formulation one had the highest RI values (0.18) whereas the RI value of formulation three (-0.01) was the lowest among the tested formulations. Formulation four showed certain repellence and oviposition deterrence effects (RI= -0.01; ODI= -44). In no-choice experiments, F3 and F4 showed a reduction in adult mean number of 34.1% and 46.9%, respectively, and accordingly there was a reduction in the mean number of laid eggs by 77.3% and 81.2%, respectively, compared with the control. Generally, there was a clear oviposition deterrent effect of F3 and F4 on SLW females and that could be used in managing SLW.

Key words: Silverleaf whitefly, Essential oil formulations, Repellence index, Oviposition deterrence index.

8.1. Introduction

Apart from its importance as a serious agricultural insect pest, the silverleaf whitefly, *Bemisia tabaci* B biotype, (Gennadius) (Hemiptera: Aleyrodidae) has developed resistance to most of the conventional insecticides (Byrne and Bellows 1991; Perring 2001; Erdogan et al. 2008). It attacks several vegetable and ornamental crops and causes severe damage resulting in reduction in production quality and quantity. It disrupts plant growth by sucking plant sap, excreting honeydew and transmitting some plant viruses including geminiviruses, closteroviruses, carlaviruses, potyviruses, nepoviruses, luteoviruses and DNA-containing rod-shaped virus (Duffus 1987; Jones 2003).

Alternative control methods to conventional insecticides such as plant essential oils have been reported that could play an important role in suppressing whitefly populations (Isman 2000). In the literature, these oils have shown several effects on whiteflies including insecticidal, repellent and /or oviposition deterrent effects. Three new plant essential oil formulations were developed and they showed promising results with an insecticidal effect against the developmental stages of the silverleaf whitefly B biotype (chapter seven). These formulations contain plant essential oils and surfactants that are used for the first time against an agricultural insect pest. These formulations were: formulation one (F1) containing mustard oil, MW-100 emulsifier, lauryl glucoside (LG) and cellosolve acetate (DEGME), formulation three (F3) containing mustard oil, MW-100 emulsifier, laureth carboxylate (LEOCS) and monoethanolamine (MEA) and formulation four (F4) containing mustard oil, MW-100 emulsifier, LG and MEA.

Previous studies showed that some essential oils have proven to be effective as repellent and oviposition deterrent products. For example, thyme (*Thymus vulgaris* L.) against red flour beetle (*Tribolium castaneum* Herbst) (Clemente et al. 2003), rose geranium (*Pelargonium graveolens* L'Her) (Geraniaceae) oil against the mite (*Leptotrombidium* sp.) (Acari: Trombiculidae) (Eamsobhana et al. 2009) and the maize weevil (*Sitophilus zeamais* Motschulsky). Many essential oils have been tested for their repellence and oviposition deterrence effects against whiteflies. Neem, *Azadirachta indica* A. Jass. (Meliaceae) oil extract is one of the most common repellent essential oils. It has been used previously against whitefly and showed promising results (Simmonds et al. 2002; de Almeida Marques et al. 2014). Pavela and Herda (2007) tested the repellent effects of pongam oil on settlement and oviposition of the common greenhouse whitefly *Trialeurodes vaporariorum*. Other essential oils from thyme (*T. vulgaris* L.), patchouli (*Pogostemon cablin* Blanco) and lemon-scented gum (*Corymbia citriodora* Hook) (Myrtaceae) have shown repellent effects against *B. tabaci* B biotype (Yang et al. 2010). Garden cress (*Lepidium*

sativum L.) (Brassicaceae) and yellow milfoil (*Achillea biebersteinii* L.) (Asteraceae) also have repellent effects against silverleaf whitefly (SLW) (Ateyyat et al. 2009). The yellow milfoil also has oviposition deterrent effects against SLW (Dehghani et al. 2012; Dehghani and Ahmadi 2013).

The repellent and oviposition deterrent effects of these new formulations need to be tested. The objectives of this study were to evaluate those behavioral parameters compared with the common repellent essential oil, neem oil, against the silverleaf whitefly. Additionally, the repellence index (RI) and oviposition deterrent index (ODI) were determined using choice and no-choice tests.

8.2. Materials and Methods

8.2.1. Whitefly and tomato seedlings

The methods for rearing whitefly and plant seedlings are described in chapter three section 3.2.1.

8.2.2. Essential oil formulations

From several preliminary tests undertaken in laboratory conditions it was found that, some surfactants and essential oils showed promising effects against the developmental stages of *B. tabaci* B biotype (chapter three). The effective products were mixed together to produce three different formulations and the sublethal effects of these formulations were tested in this experiment against the adults of SLW. The components of the formulations and the percentage proportions of each one are detailed in table 8.1.

Table 8.1: The components of the three formulations that used in this experiment and their percentages in the formulations.

Formulation 1	**Formulation 3**	**Formulation 4**	**%**
Lauryl glucoside	Laureth carboxylate	Lauryl glucoside	20
MW 100 emulsifier	MW 100 emulsifier	MW 100 emulsifier	40
Mustard oil	Mustard oil	Mustard oil	20
Cellosolve acetate	Monoethanolamine	Monoethanolamine	20

8.2.3. Adult repellence and oviposition deterrent tests

8.2.2.1. Choice test

Two hours after spraying, two plastic tubes containing leaves were located about 20 cm away from each other inside insect cages (60 cm × 60 cm × 60 cm) (Figure 8.1). The front and back panels of the cage were of clear plastic for observation of insect activity. The two side panels were of fine Polyester netting (96 x 26 mesh) for ventilation. One leaf was treated with the product whereas the other was sprayed with water as a control. Twenty five male and twenty five female adults of *B. tabaci* B biotype were released on the floor, in the centre of the cage. The whiteflies were aged between 24 and 48 h. Each cage represented one replicate. There were four replicates and all the replicates started at the same time (6:00 am). The cages were held under laboratory conditions at a temperature of 27 ± 2 °C and 60 ± 10 % relative humidity with a 14:10 (L:D) photoperiod. The numbers of whiteflies on the leaves per treatment were counted at 2, 6, 12, 24 and 48 h after release.

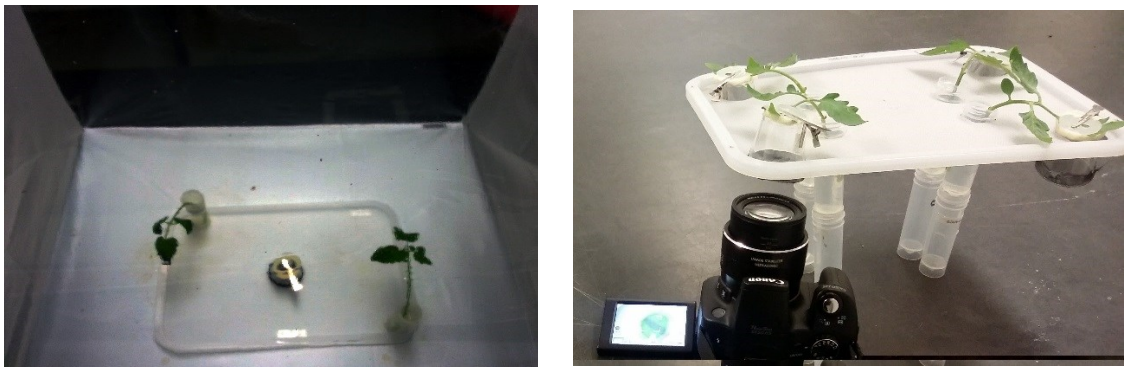


Figure 8.1: Choice test (Left), No-Choice test (Right).

8.2.2.2. No-choice repellence test

Four leaves with three apical leaflets were each sprayed with one of three formulations (F1, F3 and F4) and one with water as a negative control, as described for the choice test. Neem oil was also used as a positive control. There were four replicates (each leaflet was one replicate) for each treatment. After two hours, 15 male and 15 female adults were introduced into each clip cage (2 cm in diameter) and then the adults were exposed to the treated leaflets. Numbers of adults on the lower surface of the leaves were counted 2, 6, 12, 24 and 48 h after the adult introduction. The means with standard errors were calculated. A digital camera (Canon, Power shot, SX50 HS) was used to take photographs and then count adults to avoid disturbing the leaves (Figure 8.1). The means with

standard errors of the total number of eggs laid on leaves were counted at 48 h after the adult introduction.

8.2.4. Statistical analysis

A repellence index (Baldin et al. 2013; Baldin and Lara 2001; Schilick-Souza et al. 2011) was calculated according to the equation $RI = 2T/ (T + C)$, where T is the number of insects on the treated surface and C is the number of insects on the control surface. RI values <1 indicated repellence of *B. tabaci* by the treated leaflets as compared with the control; RI values >1 indicated attractiveness of *B. tabaci* by the treated leaflets as compared with the control. Classification of the RI was accomplished by comparing the number of adults on each sprayed leaflet with the number of adults on the leaflet containing the control, considering the standard error of the mean of the assay for differentiation (Baldin et al. 2013). After the last count of the adults, the number of eggs were counted under microscope on the apical leaflets' abaxial surface. The oviposition was also analyzed through an oviposition deterrence index (ODI), which was given by the equation $ODI = [(T - C)/(T + C)] \times 100$, where T is the number of eggs counted on the leaflet treated with the test formulation and C is the number of eggs counted on the leaflet sprayed with water as a control. ODI values vary from +100 (very attractive) to −100 (complete deterrence). Classification was conducted by comparing the number of eggs on each leaflet treated with formulations with the number of eggs on the leaflet sprayed with water, considering the standard error of the mean of the assay for differentiation (Schilick-Souza et al. 2011).

The repellence indices (RIs) value differences of products and formulations were subjected to Two-Way analysis of analysis of variance (ANOVA) using Minitab 17. Graphs of RIs and oviposition deterrence indices (ODIs) of the choice test that include the means with standard errors were presented using the Sigma Plot program. The data of the no-choice test including the mean number of adults on tomato leaves treated with formulations obtained after; 2, 6, 12, 24 and 48 h of SLW adult introduction (n=30) and mean number of eggs counted after 48 h was subjected to ANOVA. Regression analysis was used to determine relationships between RI values and time after adult exposure of the formulations. Results were assessed at the 95% confidence level and Tukey's test was used to determine the significant difference between treatments.

8.3. Results and Discussion

8.3.1. Choice test

The mean numbers of the adults of SLW on tomato leaves of the formulations, sprayed with 1.25% of the formulations, compared with the negative control (water) and with the positive control (neem oil) are presented in figure 8.2. The observations were taken at different times; 2, 6, 12, 24 and 48 h after adult introduction (AAI).

At 2 h and 6 h (8:00 am and 12:00 pm) AAI, there were almost no adults observed on the lower side of both leaves. During this time, the adults were in the process of emerging from the plastic tube or were located at the top of the cage. At 12 h (6:00 pm) AAI observation, the number of adults on both leaves had increased. 24 h and 48 h (6:00 am) AAI observations showed a reduction in the number of the adults on both leaves. This could be as a result of the time of day, with the adults being attracted to the light at the top of the cage, or because the formulations lost their repellent/attractant effects.

Neem oil (0.5%) was used as a positive control. Almost no adults were observed on the treated leaves at 2 h and 6 h AAI. However, there were a number of adults observed on the control leaves, indicating a repellent effect of the neem. At 12 h AAI, equal numbers of adults were counted on both leaves. At, 24 h and 48 h, more adults were on treated leaves with neem oil, indicating that the neem oil may have lost its effectiveness.

These data were analyzed by calculating the repellence indices (RIs) (Figure 8.3). Data were subjected to two-way ANOVA to determine the repellent/attractant effects statistically and showed that there were no significant effects of treatment or of time, respectively (F: 0.05, 0.47; P: 0.984, 0.758).

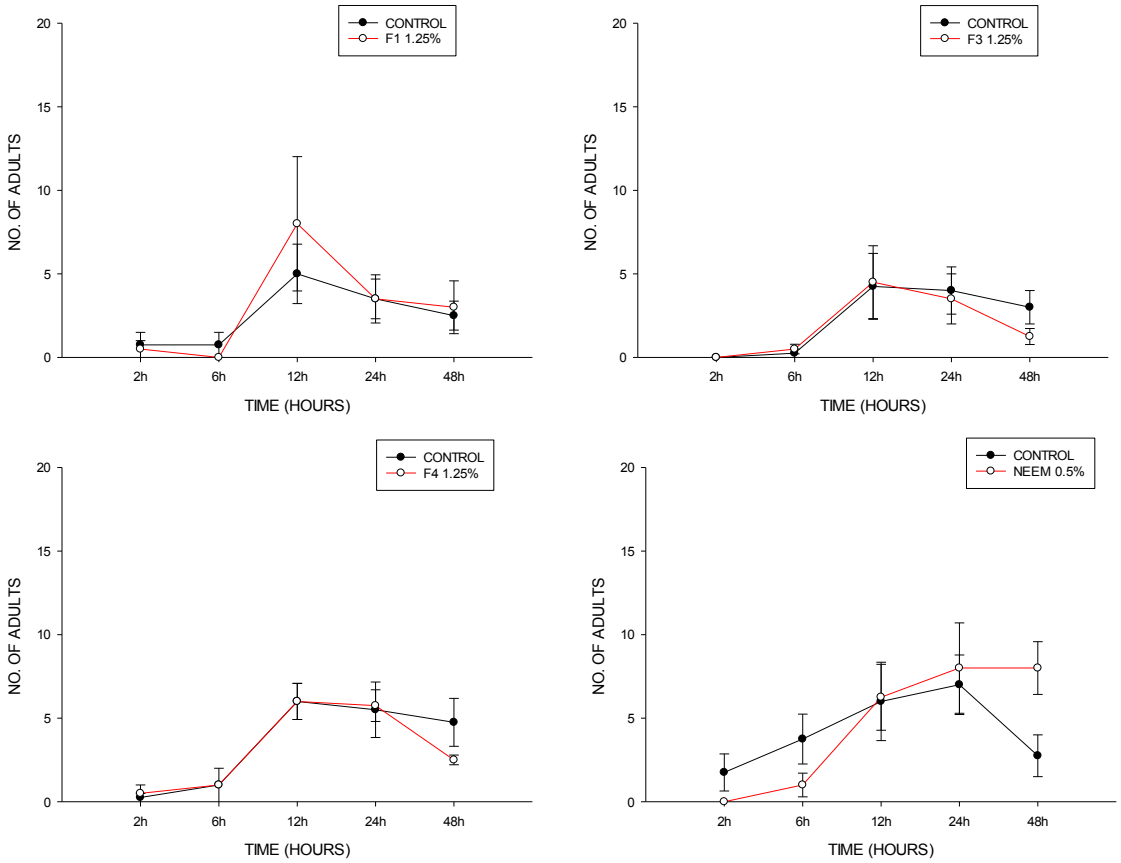


Figure 8.2: The mean numbers ± standard errors of the adults on tomato leaves treated with formulations (F1, F3 and F4) or neem oil (positive control), compared with the negative control (water) in choice test.

The formulations, F1, F3 and F4 and neem oil showed slight repellent effects at the first two observations (2 h and 6 h AAI). However, there was no repellence effect (RI ≈ 1) during observation times: 12 h, 24 h and 48 h for F1, F3 and F4 (Figure 8.3) with an exception for F3 that worked as repellent (RI = 0.54) at 48 h AAI. Neem oil showed some repellent effects at the first two observations times. After that, there was no effect at 12 h AAI. However, it worked as an attractive after 24 and 48 h AAI (Figure 8.3).

The formulations F1, F3 and F4 and neem oil were also tested to determine their oviposition deterrence against adult females of SLW. The oviposition deterrence indices (ODIs) were estimated after 48 h AAI. From figure 8.4, generally, the deterrent/attractant effect was very low for all formulations and neem oil. The average ODIs were -8.3, -9.0, 3.3 and 2.1, respectively. F1 and F3 showed some deterrent effects while F4 and neem oil showed attractant effects. Regarding the number of laid eggs, F4 may have resulted in a reduction in oviposition when the average number of laid eggs was compared between control and treated leaflets, 9.8 and 3.8, respectively (Figure 8.5).

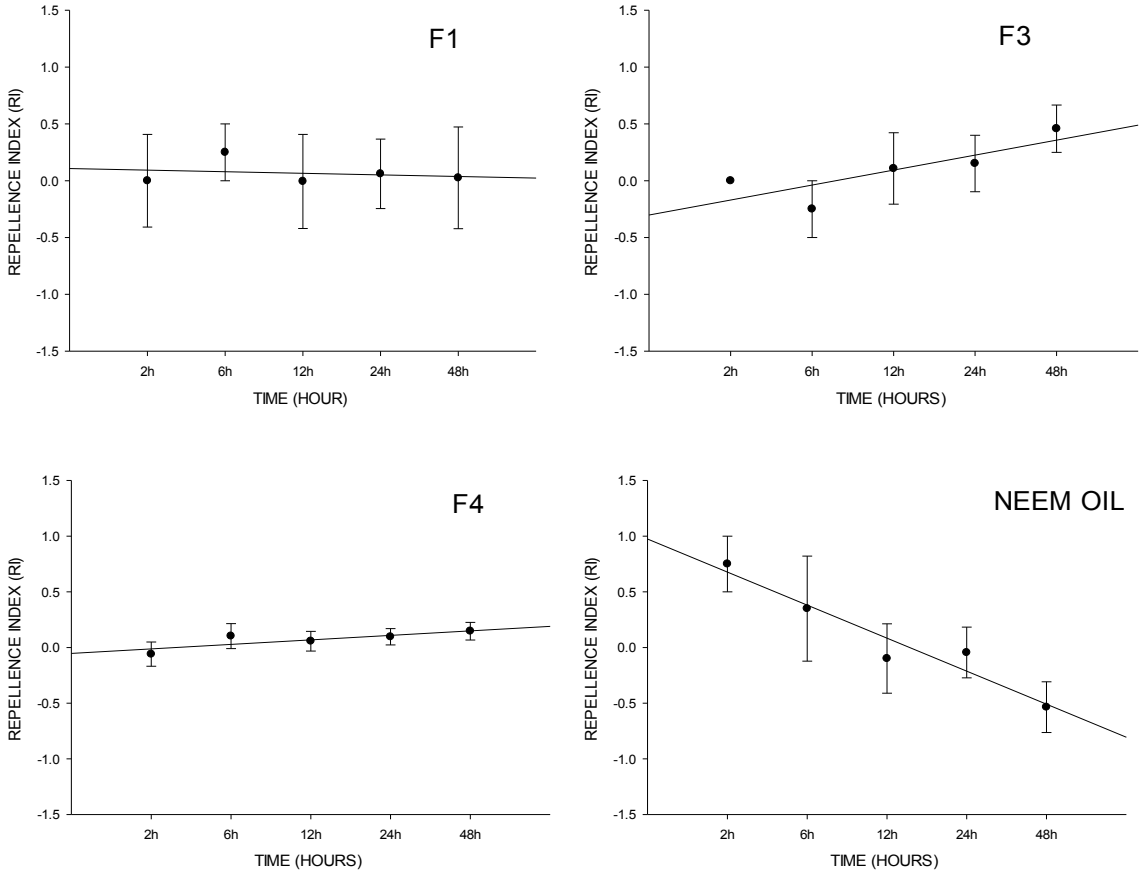


Figure 8.3: Repellent index (RI) with standard errors of tested formulations obtained after 2, 6, 12, 24 and 48 h of SLW adult introductions compared with neem oil.

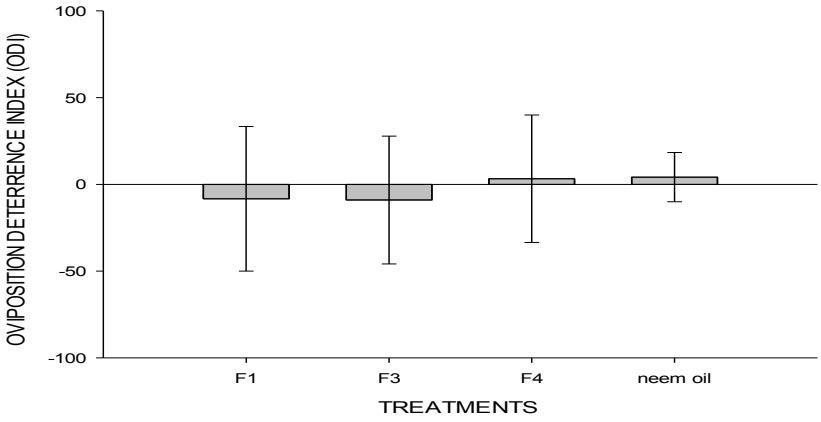


Figure 8.4: Oviposition deterrent index (ODI) with standard errors of the formulations obtained after 48 h of SLW adult introductions compared with neem oil.

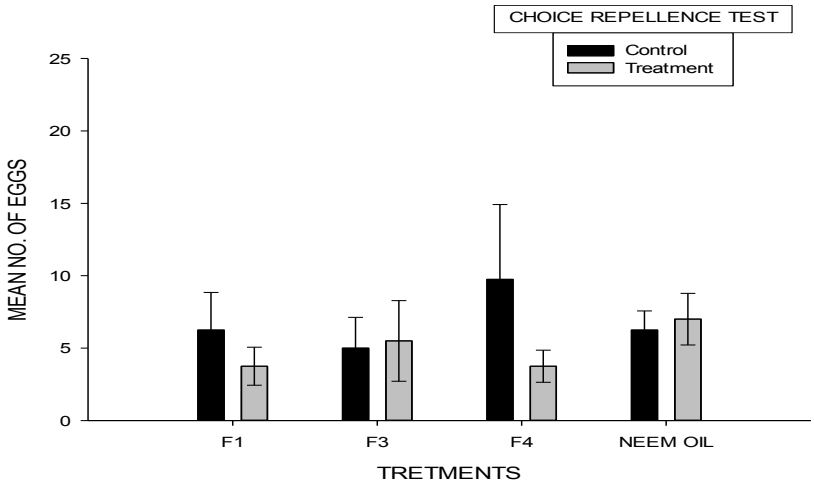


Figure 8.5: Mean numbers of SLW eggs laid after 48 h of adult exposure to the formulations and neem oil comparing with control (water).

In general, neem oil and F1 showed some repellent effects at the first 6 h AAI, whereas F3 and F4 presented some attractant effects at the same time. At 12 h and 24 h AAI, the number of SLW adults were almost equal in both leaflets, treated and control leaflets. At 48 h, the number of adults on treated leaflets were higher than on the control ones for F1 and neem oil, whereas, the leaflets treated with F3 and F4 had fewer adults than the control ones. According to Tukey's test, there were no significant differences of the RIs between the formulations at different times ($P>0.05$).

8.3.2. No-choice test

The repellent/attractant effects of the three formulations were tested against SLW adults and compared with control leaves sprayed with water in no-choice tests. Numbers of adults were observed and counted at different time intervals; 2, 6, 12, 24 and 48 h AAI. Laid eggs were counted after 48 h. Among the formulations, F1 showed the highest average number of settled adults (13.0) on the lower surface of the leaves, whereas, F4 had the lowest average number of adults (9.1) (table 8.2). However, there were no significant differences between the formulations compared with the control (17.2) according to the Tukey's test ($P>0.05$) ($F= 4.34$, $P= 0.11$).

At the first check (2 h AAI), at most 1.5 adults were observed on the lower surface of the leaves compared with 11.75 adults on the leaves sprayed with water. At that time, the adults flew away from the treated leaves inside the cages (figure 8.6). At 6 h and 12 h AAI, the number of adults on the treated leaves with the formulations increased. However, then slight decreases in adult numbers were observed at 24 h and 48 h AAI. Mean numbers of eggs were counted after 48 h AAI (Table

8.2). Although there were significantly fewer adults on leaves sprayed with F3 and F4 (34.1% and 46.9%, respectively) compared with the control this difference was not statistically significant (P>0.05). However, there were significantly fewer eggs laid on these leaves (77.3% and 81.2%, respectively) than the control (P<0.05) (Table 8.2 and figure 8.7). This suggests a deterrent effect of these formulations on the SLW females. The positive control (neem) did not have any significant effect on the number of adults or number of eggs laid on the leaves (P>0.05).

Figure 8.6: SLW adults flew away from the treated leaflets 2 h after adult introduction.

Table 8.2: Mean number of adults on tomato leaves treated with formulations obtained after; 2, 6, 12, 24 and 48 h of SLW adult introduction and mean number of eggs counted after 48 h (n=30).

Treatment	No. of Adults (Mean)					Mean*	No. of Eggs* (Mean)
	2 h	6 h	12 h	24 h	48 h		
NEEM	15.75	22.25	23.5	25	25.5	22.4[a]	79.75[a]
C	11.75	13	19.75	19.5	21.75	17.15[ab]	63.75[ab]
F1	1.5	16	19.75	14.25	13.5	13[ab]	38.00[bc]
F4	0.75	10.25	12.25	11.25	11	9.1[b]	14.50[c]
F3	0	7.5	17.5	15.75	15.75	11.3[b]	12.00[c]

* Means that share the same letter are not significantly different (Tukey's test; p = 0.05).

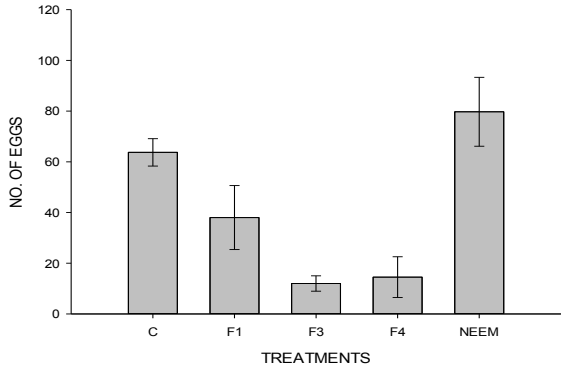


Figure 8.7: Number of SLW eggs laid after 48 h of adult exposure to tested formulations compared with negative control (water) and positive control (neem oil).

Previous studies of the sublethal (repellent, attractant and/or oviposition deterrent) effects were conducted using plant essential oils against whitefly. For example, Simmonds et al. (2002) studied the behavioural effects of two products derived from neem, *A. indica* on the glasshouse whitefly, *T. vaporariorum* and the parasitoid *E. formosa*. They found that 500 ppm did deter both the whitefly and the parasitoid adults from ovipositing. In contrast, neem in our study did not prevent SLW adults from laying eggs but showed repellent effects at the few first hours AAI in a choice test. It did not have any repellent effects in a no-choice test. This result agreed with a study by Schmutterer (1990) which showed that neem based insecticide, Margosan-O, an azadirachtin-containing formulation from neem seed extract, had a relatively weak effect on greenhouse whitefly. Another study by Lowery and Isman (1994) reported that the survival of nine species of adult aphids was unaffected by both the limonoid AZ and neem seed oil. Regarding the length of effectiveness, a study by Pavela and Herda (2007) showed that pongam oil showed relatively long-lasting repellent and oviposition deterrent effects on the adults of greenhouse whitefly *T. vaporariorum*. Its effectiveness lasted at least 12 days after application. However, in the present study, 48 h was the maximum time for the repellence observations, although, the effectiveness of the formulations generally reduced with the time even over this short period.

8.4. Conclusion

In general, these formulations were tested for the first time and there was no information in literature about their effects against agricultural pests. In choice test, neem oil and F1 had the highest RI values (RI = 0.2 and 0.18, respectively) comparing with F3 and F4 which had the lowest values (RI = 0.02 and -0.01, respectively). Regarding the time, SLW adults were highly repelled at 2 h and 6 h AAI. At the time 12 h AAI, there was the lowest repellent/attractant effects (RI = -0.07). In no-choice tests, six hours after adult introduction, the adults number were increased in the treated

leaves, which could show some repellence effect at the first hours after treatments. However, when the adults settled in the treated leaves, they did not fly away from the leaves. F3 and F4 showed reduction in adult mean number by 34.1% and 46.9%, respectively, and accordingly there was a reduction in the mean number of laid eggs by 77.3% and 81.2%, respectively, comparing with the control.

CHAPTER 9: Effect of New Plant Essential Oil Formulations on Silverleaf Whitefly Parasitoid *Eretmocerus hayati* (Zolnerowich and Rose) Emergence from Treated Silverleaf Whitefly Mummies and Adult Survival

Abstract

Biocontrol agents generally play an important role in suppressing insect pest populations. *Eretmocerus hayati* is one of the 112 parasitoids that attack silverleaf whitefly (SLW), *B. tabaci* B biotype. It lays eggs underneath the SLW nymphs, then the emerged larvae enter the nymphs and feed on them. Successful control programs were recorded in Australia, China and Egypt. The objective of this study was to assess the lethal impact of three new plant essential oil formulations (F1, F3 and F4) on the *E. hayati* parasitoid. Three different rates of the formulations were used (0.69%, 1% and 1.23%) in replicated experiments. A glass - slide bioassay method was used in this study. The data of parasitoid mortalities were calculated and then subjected to statistical analysis. Formulation one had the lowest significant adverse effect on the parasitoid among the tested formulations. It resulted in 11.1% ± 11 mortality. There were no significant differences between the tested rates on the parasitoids. Formulations three and four showed severe effects on the parasitoids. Formulation one was effective against SLW, and with minimal effects on the parasitoid, it is the most suitable formulation of those tested for use in an IPM program.

Keywords: *B. tabaci* B biotype, *E. hayati* parasitoid, new plant essential oil formulations.

9.1. Introduction

Globally, 112 species of parasitoids have been recorded attacking *B. tabaci* (Lahey and Stansly 2015). Thirteen species of them belonged to the genus *Eretmocerus* (Zolnerowich and Rose 1998). *E. eremicus* Zolnerowich and Rose, *Eretmocerus, mundus* Mercet and *Eretmocerus hayati* Zolnerowich and Rose are available commercially (Liu 2007).

The parasitoid, *Eretmocerus hayati* (Zolnerowich and Rose) is one of the 112 parasitoids of the *B. tabaci* B biotype as Lahey and Stansly (2015) reported. It was identified in 1998 (Zolnerowich and Rose 1998). *E. hayati* is a solitary parasitoid laying eggs outside and under the nymphal host. After egg hatching, the first instar larva penetrates the whitefly nymphs, feeds and pupates inside the

nymphs (Yang and Wan 2011). *E. hayati* has been used successfully in Australia (De Barro and Coombs 2009), Egypt (Abd-Rabou 2004) and China (Yang and Wan 2011). In 2004, *E. hayati* was first released in Australia and showed a general reduction in *B. tabaci* population. The overall average parasitism was 85% with 29.3% of fourth instars parasitized (De Barro and Coombs 2009). Host suitability of different instars of *B. tabaci* B biotype was studied by Yang and Wan (2011). Their study showed that *E. hayati* parasitized all nymphal instars except the late fourth instar. Young instars such as first and second instars were more parasitized than older ones.

One of the main disadvantages of pesticides is their adverse impact on the natural enemies of insect pests. For example, methomyl showed 100% mortality on *E. mundus* adults (Gonzalez-Zamora et al. 2004). Methomyl (Lannate®) and indoxacarb (Steward®) caused low mortality of *E. mundus* pupae (17.6% and 7.8% respectively), although methomyl mortality was significantly higher. In another study, results showed that the longevity and fecundity of *E. mundus* adults were reduced significantly by imidacloprid and buprofezin insecticides (Sohrabi et. al. 2013). Therefore the use of selective pesticides is important to maintain natural enemy populations in nature (Prabhaker et. al. 2007).

However, studies have found that some pesticides could be used together with the parasitoid in integrated pest management programs (IPM). An evaluation of potassium salts of a fatty acid and diafenthiuron against the adult and crawler stages of the whitefly, *Trialeurodes vaporariorum*, Westwood and adult of the parasitoids *Encarsia formosa* Gahan and *E. eremicus,* was studied by Javed and Matthews (2002). The results showed that both products were harmless to adults and pupae of both parasitoids. Whitefly mortality in the presence of the parasitoid was 87.8%, significantly higher than the mortality in the absence of *E. mundus,* Mercet (59.3%). Oxamyl (Vydate®) did not produce a significant effect on the emergence of *E. mundus* adults (Javed and Matthews 2002). These results could be explained by the fact that the parasitoid larvae were protected inside the whitefly nymphs and pupae, inadequate coverage of plant canopy and/or the timing of the application (Gonzalez-Zamora et al. 2004). Plant extracts such as neem showed a reduction in the numbers of the two parasitoids *Diaeretiella rapae*, M'Intosh, a parasite on the cabbage aphid *Brevicorine brassicae* Linnaeus and *E. mundus* after the application (Zaki 2008). It also showed negative effects on *E. warrae* after foliar spray (Kumar et al. 2005; 2008). However, extracts of *Ruta chalepensis* L., *Peganum harmala* L. and *Alkanna strigosa* Boiss. and Hohen were not harmful to *B. tabaci* parasitoid, *E. mundus* (Al-mazra'awi and Ateyyat 2009). *Melia azedarach* L. fruit extracts and the parasitoid *Eretmocerus rui* Zolnerowich and Rose were compatible in controlling *B. argentifolii* (Abou-Fakhr Hammad and Mcauslane 2006). In this study, the lethal

effects of new plant essential oil formulations were evaluated against *E. hayati,* one of the key parasitoids of silverleaf whitefly, *B. tabaci* B biotype.

9.2. Materials and Methods

9.2.1. Whitefly and tomato seedlings

The methods for rearing whitefly and plant seedlings are described in chapter three section 3.2.1.

9.2.2. Essential oil formulations

The components of the formulations and the percentage proportions of each one were as in chapter eight, table 8.1. The formulations were tested at different rates, 0.69%, 1% and 1.23% compared with control (water).

9.2.3. Parasitoid, *Eretmocerus hayati*

The parasitoids, *E. hayati* were obtained from the Bugs for Bugs Company (Mundubbera, Queensland, Australia). The wasps were provided in a small plastic vials covered with a cotton plug. The wasps were released in tomato seedlings infested with the developmental stages of SLW inside 45 x 45 x 45 cm cages in an insectary in the University of Queensland, Gatton Campus maintained at 27 ± 2°C, RH 60 ± 10%, and 14:10 (Light: Dark) photoperiod.

9.2.4. *E. hayati* mortality procedure under laboratory conditions

The parasitoid wasps were released and left to parasitize the SLW nymphs. After two weeks, tomato leaves were removed from the seedlings and the parasitized nymphs were counted. The parasitized nymphs turned yellow brown in color. A glass slide bioassay (Avery et al. 2004) was used to determine the parasitoid mortality rates. Three droplets (10 µl each) of each of the prepared diluted solutions of formulations and control were placed on a glass slide. The parasitized nymphs were removed and five were placed on each droplet and then left to dry in a laminar hood for 30

minutes (Figure 9.1, left). After that, the slides were placed in petri dishes lined with wet filter paper under laboratory conditions (24 ± 2ºC and 14 photoperiod) inside cages (Figure 9.1, right). There were eight replicates (each petri dish containing 15 parasitised nymphs counted as a replicate). Mortality rates were calculated 48 h after treatment. The shriveled and/or discolored nymphs were considered dead.

Figure 9.1: Treated parasitized nymphs in the laminar hood (left) and then transfer into cages in the laboratory (right).

9.2.6. Statistical analysis

Mortality rates from all tested concentrations of formulations against the SLW parasitoid were analyzed using Minitab 17 through General Linear Model ANOVA. Tukey's test was used to identify treatments and tested rates that had significant differences between them. Regression analysis was used to determine relationships between percentages of mortality and tested rates of the formulations. Results were assessed at the 95% confidence level. Mortality rates with standard errors were presented using the Sigma Plot program.

9.3. Results and discussion

The line graphs presented in figure 9.2 summarise the effect of three new plant essential oil formulations at different tested rates against the silverleaf whitefly parasitoid, *E. hayati*. It was clear that formulation one had the lowest lethal impact on the parasitoid compared with the other two formulations. It caused mortalities at 0.69%, 1% and 1.23% of 6.7%, 9.2% and 17.5%, respectively. However, formulations three and four had a severe impact, producing high mortality rates. For

example, formulation three resulted in 25.8% mortality at 0.69% and 80% of the parasitoids were killed by tested rates 1% and 1.23%. Formulation four resulted in high mortality at all three tested rates (0.69%, 1% and 1.23%). The mean mortalities were 53.3%, 64.2% and 70%, respectively. Dead parasitized nymphs were dried, flattened and discolored from pale yellow to dark brown.

When the data were subjected to ANOVA (Table 9.1), there were significant differences amongst the treatments ($F= 11.69$, $P= 0.02$). Formulation one showed the least mean mortality rate (11.1% ± 11) and this was significantly lower than the mortalities of formulations three and four, which were 61.9% (± 11) and 62.5% (± 11), respectively ($P<0.05$). There were no significant differences ($F= 2.84$, $P= 0.17$) between the tested rates in their effect on the parasitoids. From the preliminary studies (chapter three), monoethanolamine, one of the components of formulations three and four, showed high mortality rates against SLW adults. This could explain why those formulations showed severe damage to the parasitoids.

Plant extracts showed both positive and negative effects on parasitoids. Several studies have evaluated the impact of plant extracts on parasitoids. For example, Simmons and Shaaban (2011) found that, the biorational insecticides: jojoba oil, Biovar and Neemix had the least effect on abundance of the natural enemies including *Eretmocerus* spp. in comparison with other insecticides during a 14 day evaluation period. Another study showed that when ten droplets (10 µl each) from each extract were placed on a sterile glass slide as two rows on each side of the slide and *B. tabaci* parasitized pupae were placed individually on each droplet, the mortality of *E. mundus* was 24, 12 and 8% for *R. chalepensi*s, *P. harmala* and *A. strigosa*, respectively (Al-mazra'awi and Ateyyat 2009). Conversely, Kumar et al. (2008) showed that neem oil caused high mortality rates when it was sprayed against SLW nymphs parasitized with *E. warrae*.

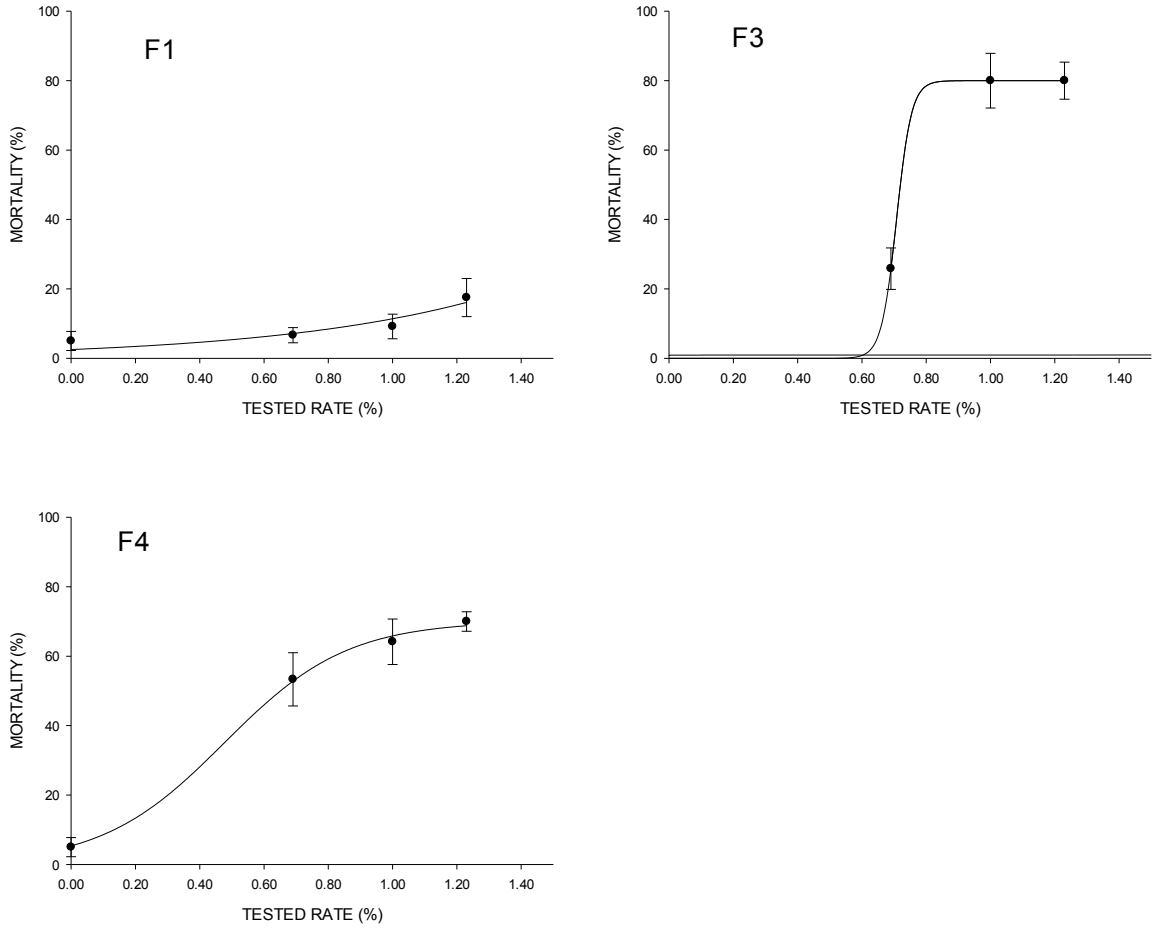


Figure 9.2: Mean mortality rates of plant essential oil formulations at different tested rates against SLW parasitoid, *E. hayati*.

Table 9.1. Mean mortality rates of three formulations at different tested rates against *E. hayati*.

Factors	Parasitoid mortality % ± SE
Formulations	
C	5.0 ± 19b
F1	11.1 ± 11b
F3	61.9 ± 11a
F4	62.5 ± 11a

Means that share the same letter are not significantly different (Tukey's test; p = 0.05).

9.4. Conclusion

There were no previous studies of these formulations in literature. Some plant extracts like neem showed a reduction in the numbers of the two parasitoids *D. rapae*, and *E. mundus* and caused high mortality rates against *E. warrae* (Kumar et al. 2008). In the current study, formulation one had a toxicity effect against all developmental stages of SLW and was found to be soft on the parasitoid,

the parasitoid reduction was 11%, whereas formulations three and four reduced the parasitoid by more than 60%. Therefore, formulation one could play a part in the IPM programs of the SLW.

CHAPTER 10: General discussion and conclusion

This research aimed to investigate how to use biopesticides to achieve not only maximum effectiveness against SLW but also minimise the off-target effects against its parasitoid, *E. hayati*. A series of experiments was carried out to assess the efficacy of the different formulations on the eggs, immature stages and adults of the silverleaf whitefly (SLW), *B. tabaci* B biotype. In this study, more than 30 new products including surfactants, essential oils, and formulations were tested against the developmental stages of SLW: egg, nymphal and adult stages. Generally, there was no single product that was effective against all the developmental stages except the mixture of mustard oil and liquid soap. However, some products, such as the surfactants, were highly toxic to nymphs and some, such as amines, were highly effective against adults.

The egg stage of the insect pests is very difficult to control, depending on where the eggs were laid by the female and also if they were covered by frass or inserted into the plant tissue. In the case of SLW eggs, it has a pedicle and it is inserted directly into a slit made in the leaf tissues by the female ovipositor. During the insertion, a glue-like substance is secreted by the colleterial gland and surrounds the pedicel protecting it. Additionally, the outer surface of the egg is smooth, making it harder for liquids, including insecticides, to adhere to the surface. Therefore, both factors make the egg stage difficult to control with insecticides. However the mixture of mustard oil and liquid soap showed excellent results against eggs. At 0.25% concentration, the mortality rate was 95.3%. The formulations also showed promising results. At a concentration of 1.23%, mortalities of the F1, F3 and F4 formulations were 85%, 70.8% and 69.2%, respectively. Nymphs were only able to partially emerge and then died. This was due to a disruption of embryogenesis and/or an effect of essential oils on newly emerged nymphs after eclosion from viable eggs, probably when they contacted with the residues on the egg chorion. These observations were consistent in egg mortality symptoms with the results of Naranjo and Ellsworth (1999) and Yang et al. (2010).

The nymphal stage consists of four instars that are all immobile except for the newly hatched nymphs, which crawl on the leaf surface while searching for a suitable settling site. Surfactants such as glucosides, showed significant effects on the nymphal instars. Younger nymphs were more sensitive to the surfactant than the older ones. At 0.25%, younger nymph mortalities of capryl glucoside (CG) and decyl glucoside (DG) were very high: 84.2% and 94.6%, respectively. In comparison, the effects of lauryl glucoside (LG) (57.9%) and lauryl sucroside (LS) (63.4%) were lower. Higher concentrations were required for older nymphs; at 1.5%, mortalities of older nymphs treated with CG, DG, LG and LS were 72.5%, 80.83%, 71.67% and 73.86%, respectively. Additionally, when the three formulations were tested against the different stages of the nymphs,

the mortality results were similar to these surfactants. The nymphs became discoloured, dried and flattened due to dehydration as a result of the disruption of the external waxy layer. It is known that surfactants can be effective for managing insect pests. For instance, Liu and Stansly (2000) studied the insecticidal activities of four surfactants (Cide-kick, Silwet L-77, M-Pede and APSA-80) and found that they had good potential for controlling *B. argentifolii*. Also, insecticidal soaps are commonly available for a wide number of pests; they also are thought to work by disrupting the external waxy layer of insects or disrupting cell membranes.

The adult stage was more sensitive to the amines. At 0.25%, monoethanolamine (MEA), monoisopropanolamine (MIPA) and diisopropanolamine (DIPA) mortality rates were 77.8%, 82.5% and 43.1%, respectively with low phytotoxicity effect. There was a significant effect on adults ($P < 0.001$) according to probit analysis with 95% confidence interval. No previous studies were found.

Paes et al. (2012) reported that synthetic mustard essential oil (SMEO) (90% AITC) can affect the developmental stages of the maize weevil (*Sitophilus zeamais*). Similarly mustard oil from *Brassica alba* found detrimental against the cotton leafworm, *Spodoptera littoralis* (Abd El-Aziz and Sharaby 1997). Additionally ITCs was found to be toxic against the eggs of *Dasineura brassicae* (Ahman 1985) and AITC from commercial source also found toxic to the red flour beetle *Tribolium castaneum* as fumigant (Santos et al. 2011). Wolfson (1982) observed that developmental responses of some insects to *Brassica nigra* were due to GLs compounds. Interestingly ITCs inhibited both *in vitro* germination and subsequent growth of the insect pathogenic fungus *Metarhizium anisopliae* and its ability to infect *P. cochleariae* (Inyang et al.1999). It is positive from a plant health perspective that natural enemies may also benefit from the glucosinolate–myrosinase system in search of hosts by using volatiles, such as ITCs, emitted from infested *Brassica* plants as cues (Pope et al. 2008). All of the tested formulations (F1, F3 and F4) in this project showed highly significant effects on eggs and nymphs (≈70%) whereas they caused moderate adult mortality rates (≈ 40%).

Repellence and oviposition deterrence effects of the formulations were examined in choice and non-choice tests against SLW adults determining repellent index (RI) and oviposition deterrent index (ODI). From the results of choice tests, F1 had the highest RI values (0.18) whereas the RI value of F4 (-0.01) was the lowest among the tested formulations. The F4 had the most repellent effect on the SLW adults, whereas F1 did not appear to have any repellent activity. F4 also showed certain oviposition deterrence effects (ODI= -44). In no-choice experiments, F3 and F4 showed a reduction in adult mean numbers on treated leaves by 34.1% and 46.9%, respectively, and accordingly there were a reduction in the mean number of laid eggs by 77.3% and 81.2%, respectively, compared with the control.

Previous studies showed potential repellent effects of essential oils such as neem, *A. indica* against SLW (de Almeida Marques 2014; Simmonds et al. 2002). In a choice test, tomato leaves treated with the essential oil extracted from patchouli (*P. cablin*) showed 69.3% fewer SLW than on the control leaves at 24 h after release and 74.5% fewer eggs (Yang et al. 2010). Similarly, *Pimpinella anisum* L., *Galium longifolium* Sibth. and SM., *Retama reatam* Raf. and *Ballota ondulata* Sieber and Fresen exhibited repellent effects against adult whiteflies in studies by Ateyyat et al. (2009). The yellow milfoil was also found to have oviposition deterrent effects against SLW (Dehghani et al. 2012; Dehghani and Ahmadi 2013).

When the insecticidal effect of these formulations was evaluated against one of the key SLW parasitoids (*E. hayati*), F1 had the lowest significant adverse effect on the parasitoid (11.11%). There were no significant differences between the tested rates on the parasitoids. F3 and F4 showed severe effects on the parasitoids, 61.94% and 62.50%, respectively, that could be due to one of their components: Monoethanolamine showed, in the preliminary tests, high mortality rates on the SLW adults and that might also affect the SLW parasitoid. From previous studies, some essential oils like neem oil had adverse effects on whitefly parasitoid (Zaki 2008). However, extracts of *Ruta chalepensis* L., *Peganum harmala* L. and *Avena strigosa* Schreber were not harmful to *B. tabaci* parasitoid**,** *E. mundus* (Al-mazra'awi and Ateyyat 2009).

The toxicity of some surfactants were studied for the first time against the developmental stages of the silverleaf whitefly such as glucosides (CG, DG, LG and LS) and amines such as (MEA, MIPA and DIPA). The glucoside surfactants were very effective against the nymphal stages especially the younger ones. The amines showed higher toxicity on SLW adults. A mixture of mustard oil and liquid soap at the ratio 3:1 at 0.25% v/v showed toxicity effects on eggs (95.8%), young nymphs (86.4%), old nymphs (47.4%) and adults (34%). These surfactants and mustard oil could be used in future in new biopesticide formulations.

Five formulations were prepared from the different effective surfactants and essential oils in order to control all the developmental stages of SLW in one spray. Four of the formulations included mustard oil whereas the fifth included neem oil to be used as a positive control. However, the formulation containing neem oil showed severe phytotoxicity at a lower tested rate (0.44%) on tomato leaves. This was an unexpected result because neem oil is considered to be mild and can be used in higher testing rates against insect pests (Pinheiro et al. 2009). Another formulation containing laureth carboxylate, MW 100 emulsifier, mustard oil and cellosolve acetate also showed severe phytotoxicity and so was excluded from further testing. The remaining three formulations (see chapter seven for their components) were tested for toxicity against all developmental stages of SLW and one of its main parasitoids, *E. hayati*. The formulation repellent and oviposition deterrent

effects were also estimated against the SLW adults. These formulations contain mustard oil as an essential oil, MW-100 as an emulsifier and lauryl glucoside, laureth carboxylate, cellosolve acetate and/or monoethanolamine as surfactants. Because of the phytotoxicity effects, the formulation rates could not exceed 1.25% v/v.

Mustard oil showed lethal effects against all developmental stages of SLW whereas the surfactants such as glucosides and amines like monoethanolamine were very effective against the nymphal and adult stages, respectively. Therefore, the three formulations prepared from these products were expected to combine these effects. The toxicity effect of the formulations was in a range of 60% - 70% against egg and nymphal stages, but was less effective (30% - 40%) when the SLW adults were exposed to the formulations under laboratory conditions. When the adults were directly sprayed by the formulations under glasshouse conditions, the mortality was higher (70% – 85%) . However, the formulations F3 and F4, at 1.25% v/v had repellent and egg laying deterrent effects on the adults in a no-choice test. Unfortunately F3 and F4 also had adverse effects on the parasitoid, *E. hayati* (60% mortality) whereas F1 had the lowest mortality rate (11.11%).

Formulation one contained lauryl glucoside (20%), mustard oil (20%), cellosolve acetate (20%) and MW-100 emulsifier (40%). Under laboratory conditions it showed high toxicity effects on the developmental stages of *B. tabaci* and low effects on its parasitoid, *E. hayati* compared with the other two formulations. Formulation one therefore appeared to be a promising option and could play an important role in managing the SLW populations and be a part of integrated pest management (IPM) programs. Most conventional insecticides used to manage insect pests of the cultivated crops are broad spectrum, controlling the target insect pests and the non-target natural enemies as well. But substances obtained from plant resources have been generally considered safe compared to the conventional insecticides. However, not all substances extracted from plants are always safe. Safety aspect of plant extracted substances is very important since there are many beneficial insects which contribute highly to controlling insect pests (Raguraman 2009; Koul and Wahab 2004; Schmutterer 1992). Different kinds of beneficial insects play a very important role in natural control of insect pests. Conservation of beneficial insects is achieved by using IPM practices that allow beneficial insects to survive using plant extracts as insecticides.

Under glasshouse conditions, the formulations showed better effects against the adult stage than egg and nymphal stages compared with their effects under laboratory conditions. The average mortality rates of the formulations (F1, F3 and F4) when sprayed in the glasshouse were 71.8%, 73.1% and 86.2%, respectively. However, the adult mortality was less than 20% under laboratory conditions at

the same tested rates. The difference between the two trials was that in the laboratory, the adult were directly exposed to wet leaflets whereas in the glasshouse the adults were directly subjected to the formulations through spraying. This indicates that the formulations have contact insecticidal effect. In both cases the reason for reduction in egg and nymph mortalities could be that these developmental stages did not make good contact with the sprayed formulations.

This research showed that when the formulations were tested against the parasitoid of the SLW (*E. hayati*), F1 was very soft on the parasitoid and it had the lowest mortality rate (11.11%) comparing with the other two formulations (60%). It was also effective against SLW, and with minimal effects on the parasitoid it is the most suitable formulation of those three tested for use in an IPM program.

For future work, this project showed potential insecticidal activities of the formulations against SLW developmental stages. However, the percentages of the formulation components need to be adjusted to minimise the phytotoxicity and enhance the toxicity of the formulations. Also more studies need to be conducted testing the formulation one against the immature stages of the parasitoid, *E. hayati* in laboratory and field trials. Additionally, more experiments are required to evaluate the formulations under field conditions against SLW and other agricultural insect pests.

References

Abd El-Aziz, S & Sharaby, M 1997, 'Some biological effects of white mustard oil, *Brassica alba* against the cotton leafworm, *Spodoptera littoralis* (Boisd.)', *Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz*, vol. 70, no. 3, pp. 62-4.

Abd-Rabou, S 2004, 'Biological control of *Bemisia tabaci* Biotype "B" (Homoptera: Aleyrodidae) by introduction, release and establishment of *Eretmocerus hayati* (Hymenoptera: Aphelinidae)', *Journal of Pest Science*, vol. 77, no. 2, pp. 91-4.

Addor, RW 1995,' Insecticides', In CRA Godfrey (Balke & Diosady) *Agrochemicals from natural products*, Marcel Dekker, New York, pp 1–62.

Abou-Fakhr Hammad, E & Mcauslane, HJ. 2006, 'Effect of *Melia azedarach* L. Extract on *Bemisia argentifolii* (Hemiptera: Aleyrodidae) and Its Biocontrol Agent *Eretmocerus rui* (Hymenoptera: Aphelinidae) ', *Environmental Entomology,* vol. 35, no. 3, pp. 740 – 745.

Agrawal, A, Tuzun, S & Bent, E 1999, *Induced Plant Defenses against Pathogens and Herbivores*. St. Paul, MI: APS Press.

Ahmad, M, Arif, MI, Ahmad, Z & Denholm, I 2002, 'Cotton whitefly (*Bemisia tabaci*) resistance to organophosphate andpyrethroid insecticides in Pakistan', *Pest Management Science*, vol. 58, no. 2, pp. 203-8.

Åhman, I 1985, 'Toxicities of host secondary compounds to eggs of the *Brassica* specialist *Dasineura brassicae*', *Journal of Chemical Ecology*, vol. 12, pp. 1481-1488.

Ahmed, M 2007.' Potentiation/antagonism of pyrethroids with organophosphate insecticides in *Bemisia tabaci* (Homoptera: Aleyrodidae)', *Journal of Economic Entomology*, vol. 100, no.3, pp. 886-893.

Ahmed, UAM, Bashier, NHH & Muafi, K. 2011,' Evaluation of insecticidal potentiality of aqueous extracts from *Calotropis procera* Ait. Against *Henosepilachna elaterii* Rossi. 469 pp. In Global Conference on Entomology, March 5-9, 2011, Chiang Mai, Thailand. Century Foundation, India.

Al Lawati, HT, Azam, KM, Deadman, ML 2002, ' Potential of Omani flora as source of natural products for control of pulse beetle, *Callosobruchus chinensis*' , *Agricultural Sciences*, vol. 7, no.1, pp. 59-63.

Al-mazra'awi, M & Ateyyat, MA 2009.' Insecticidal and repellent activities of medicinal plant extracts against the sweet potato whitefly, *Bemisia tabaci* (Hom.: Aleyrodidae) and its parasitoid *Eretmocerus mundus* (Hym.: Aphelinidae)', *Journal of Pest Science*, vol. 82, pp. 149-154.

Al-Musa, A 1982, 'Incidence, economic importance, and control of tomato yellow leaf curl in Jordan', *Plant Diseases*, vol. 66, pp. 561–563.

Allaby, M 2013,' A Dictionary of Plant Sciences (3ed), Oxford University Press, viewed 5 November 2014, <http://www.oxfordreference.com.ezproxy.library.uq.edu.au/view/10.1093/acref/9780199600571.001.0001/acref-9780199600571-e-284?rskey=X4yIUv&result=292>.

Alvarez, P & Abud-Antun, A 1995, 'Reporte de Republica Dominicana.',*Ceiba (Honduras)*, vol. 36, pp. 39–47.

Aly, R, Ravid, U, Abu-Nassar, J, Botnick, I, Lebedev, G, Gal, S, Ziadna, H, Achdari, G, Smirov, E, Meir, A, & Ghanim, M 2011,'Biological activity of natural phytoecdysteroids from *Ajuga iva* against the sweetpotato whitefly *Bemisia tabaci* and the persea mite *Oligonychus perseae*', *Pest Management Science,* vol. 67, pp.1493-8.

APCJ 2006, 'Surfonic MW 100: new emulsifier from Huntsman', 2006, *Focus on Surfactants*, vol. 2006, no. 5, pp. 4-5.

Arnó, J, Gabarra, R, Liu, T-X, Simmons, AM & Gerling, D 2010, 'Natural Enemies of *Bemisia tabaci*: Predators and Parasitoids', in PA, Stansly & SE. Naranjo (eds.), Bemisia*: Bionomics and Management of a Global Pest*, Springer Science+Business Media, pp. 385-421.

Arno, J, Roig, J & Riudavets, J 2008,' Evaluation of *Orius majusculus* and *O. laevigatus* as predators of *Bemisia tabaci* and estimation of their prey preference', *Biological Control*, vol. 44, pp. 1-6.

Aslan, İ, Özbek, H, Çalmaşur, Ö & Şahin, F 2004, 'Toxicity of essential oil vapours to two greenhouse pests, *Tetranychus urticae* Koch and *Bemisia tabaci* Genn', *Industrial Crops and Products*, vol. 19, no. 2, pp. 167-73.

Ateyyat, MA, Almazra'awi, M, Abu-Rjai, T & Shatnawi, A 2009, ' Aqueous extracts of some medicinal plants are as toxic as lmidacloprid to the sweet potato whitefly, *Bemisia tabaci*', *Journal of Insect Science*, vol. 9, no. 15, pp. 1-6.

Attique, MR, Rafiq, M, Abdul Ghaffar, Zahoor Ahmad & Mohyuddin, AI 2003,' Hosts of *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae) in cotton areas of Punjab, Pakistan', *Crop Protection*, vol. 22, pp.715–720.

Avery, PB, Faull, J & Simmonds, MSJ 2004, 'Effect of different photoperiods on the growth, infectivity and colonization of Trinidadian strains of *Paecilomyces fumosoroseus* on the greenhouse whitefly, *Trialeurodes vaporariorum*, using a glass slide bioassay', *Journal of Insect Science*, vol. 4, no. 38, pp. 1-10.

Azab, AK, Megahed, MM & El-Mirsawi, DH 1971,' On the biology of *Bemisia tabaci* (Genn.)', *Bulletin of the Entolomological Society of Egypt*, vol. 55, pp. 305-15.

Bailey, A, Chandler, D, Grant, WP, Greaver, J, Prince, G & Tatchell, M 2011, *Biopesticides: pest management and regulation*, CABI Publishing,

Bailey, MA 1930,'Leaf curl disease of cotton in the Sudan', *Bulletin of Entomological Research*, vol. 21, pp. 280–288.

Baldin, ELL & Lara, FM 2001, 'Attractiveness and leaf consumption by adults of *Diabrotica speciosa* (Germ.) (Coleoptera: Chrysomelidae) in different squash genotypes', *Neotropical Entomology*, vol. 30, pp. 675–679.

Baldin, ELL, Crotti, AEM, Wakabayashi, KAL, Silva, JPGF, Aguiar, GP, Souza, ES, VenezianiRCS & Groppo, M 2013, 'Plant-derived essential oils affecting settlement and oviposition of *Bemisia tabaci* (Genn.) biotype B on tomato', *Journal of Pest Science*, vol. 86, pp. 301–308.

Baldin, EL, Aguiar, G, Fanela, TM, Soares, ME, Groppo, M & Crotti, AM 2015, 'Bioactivity of *Pelargonium graveolens* essential oil and related monoterpenoids against sweet potato whitefly, *Bemisia tabaci* biotype B', *Journal of Pest Science*, vol. 88, no. 1, pp. 191-9.

Balke, DT & Diosady, LL 2000, 'Rapid aqueous extraction of mucilage from whole white mustard seed', *Food Research International*, vol. 33, no. 5, pp. 347-56.

Bart, H-J 2011, Extraction of natural products from plants – an introduction, in H-J Bart & S Pilz (eds), *Industrial scale natural products extraction*, Wiley-VCH Verlag GmbH & Co. KGaA. pp. 1-26.

Başer, KHC & Demirci, F 2007, 'Chemistry of Essential Oils', in: R. G. Berger (ed.), *Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability*, Springer, Berlin, pp. 43-86.

Baser, KHC & Buchbauer, G 2015, '*Handbook of Essential Oils: Science, Technology, and Applications*', Second edition, Taylor & Francis.

Belay, DK, Huckaba, RM, Ramirez AM, Rodrigues, JCV & Foster, JE 2012,' Insecticidal control of *Bemisia tabaci* (Hemiptera: Aleyrodidae) transmitting Carlavirus on soybeans and detection of the virus in alternate hosts', *Crop Protection*, vol.35. pp. 53-57.

Bellows, TS JR, Perring, TM, Gill, RJ & Headrick, DH 1994, ' Description of a species of *Bemisia* (Homoptera: Aleyrodidae)', *Annals of the Entomological Society of America*, vol. 87, no.2, pp.195-206.

Bentz, JA, Reeves III, J, Barbosa, P & Francis, B 1995,' Nitrogen fertilizer effect on selection, acceptance, and suitability of *Euphorbia pulcherrima* (Euphorbiaceae) as a host plant to *Bemisia tabaci* (Homoptera: Aleyrodidae)', *Environmental Entomology*, vol. 24, pp. 40–45.

Betz, FS, Hammond, BG & Fuchs, RL 2000,' Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests', *Regulatory Toxicology and Pharmacology,* vol. 32, no.2, pp. 156-173.

Bezerra, MA, De Oliveira, MV & Vasconcelos, SD 2004,' Does the presence of weeds affect *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) infestation on tomato plants in a semi-arid agro-ecosystem', *Neotropical Entomology,* vol. 33, no.6, pp.769-775.

Bi, JL, Lin, DM, Lii, KS & Toscano, NC 2005,' Impact of cotton planting date and nitrogen fertilization on *Bemisia argentifolii* populations', *Insect Science*, vol. 12, pp. 31-36.

Björkman, M, Klingen, I, Birch, AN, Bones, AM, Bruce, TJ, Johansen, TJ, Meadow, R, Mølmann, J, Seljåsen, R, Smart, LE & Stewart, D 2011, ' Phytochemicals of Brassicaceae in plant protection and human health-influences of climate, environment and agronomic practice', *Phytochemistry,* vol. 72, pp. 538-56.

Bosco, D & Caciagli, P 1998,' Bionomics and ecology of *Bemisia tabaci* (Sternorrhyncha: Aleyrodiadae) in Italy', *European Journal of Entomology*, vol. 95, pp.519-527.

Brier, H, McLennan, A & Dougall, A 2007,' IPM in coastal soyabeans and beyond, In: Proceedings of the 14th Australian soybean conference, Bundaberg, Qld, pp. 12-18.

Brier, HB, Murray, DAH, Wilson, LJ, Nicholas, AH, Miles, MM, Grundy, PR & McLennan, AJ 2008,' An overview of integrated pest management (IPM) in north-eastern Australian grain farming systems: Past, present and future prospects', *Australian Journal of Experimental Agriculture*, vol. 48, no.12, pp.1574-1593.

Brown, JK, Frohich, DR & Rosell, RC 1995,' The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex?', *Annual Review of Entomology*, vol. 40, pp.511-34.

Brown, JK. & Bird, J 1992, 'Whitefly-transmitted geminiviruses and associated disorders in the Americas and the Caribbean Basin', *Plant Disease*, vol. 76, pp. 220–225.

Brück, E, Elbert, A & Fischer, R 2009,' Movento®, an innovative ambimobile insecticide for sucking insect pest control in agriculture: biological profile and field performance', *Crop Protection*, vol. 28, pp. 838-844.

Buckle, R 2003,' Aromatherapy in the USA', *International journal of aromatherapy, vol.* 13, no. 1, p. 42.

Buckner, JS, Hagen, MM, & Nelson, DR 1999, 'The composition of the cuticular lipids from nymphs exuviae of the silverleaf whitefly, *Bemisia argentifolii*', *Comparative Biochemistry* and *Physiology,* vol. B 124, pp. 201-207.

Buckner, JS, Freeman, TP Ruud, RL, Chu, CC & Henneberry, TJ 2002,' Characterization and functions of the whitefly egg pedicel', *Archives of Insect Biochemistry and Physiology*, vol. 49, no.1, pp.22-33.

Butler, GD Jr, Henneberry, TJ & Clayton, TE 1983,' *Bemisia tabaci* (Homoptera: Aleurodidae): development, oviposition and longevity in relation to temperature', *Annals of the Entomological Society of America*, vol. 76, pp.310-13.

Byrne, D & Bellows, TS 1991, ' Whitefly biology, *Annual Review of Entomology*, vol. 36, pp. 431–457.

Byrne, DN & Miller, WB 1990, 'Carbohydrate and amino acid composition of phloem sap and honeydew produced by *Bemisia tabaci* ', *Journal of Insect Physiol*ogy, vol. 36, no.6, pp. 433-39.

Byrne, FJ, Castle, S, Prabhaker, N & Toscano, NC 2003,' Biochemical study of resistance to imidacloprid in B biotype *Bemisia tabaci* from Guatemala', *Pest Management Science*, vol. 59, pp. 347-352.

Byrne, FJ, Oetting, RD, Bethke, JA, Green, C & Chamberlin, J 2010,' Understanding the dynamics of neonicotinoid activity in the management of *Bemisia tabaci* whiteflies on poinsettias', *Crop Protection*, vol. 29, pp. 260-266.

Çalmaşur, Ö, Aslan, İ & Şahin, F 2006, 'Insecticidal and acaricidal effect of three Lamiaceae plant essential oils against *Tetranychus urticae* Koch and *Bemisia tabaci* Genn', *Industrial Crops and Products*, vol. 23, no. 2, pp. 140-6.

Camacho, F, Sanchez, S & Pacheco, R 1997,' Absorption of carbon dioxide at high partial pressures in 1-amino-2-propanol aqueous solutions considerations of thermal effects', *Industrial and Engineering Chemistry Research*, Vol. 10, pp. 4358−4364.

Carver, M & Reid, IA 1996,' Aleyrodidae (Hemiptera: Sternorrhyncha) of Australia- systematic catalogue, host plant spectra, distribution, natural enemies and biological control', *CSIRO Division of Entomology. Technical Paper,* No. 37, pp. 55.

Castle, S & Naranjo, SE 2009,' Sampling plan, selective insecticides and sustainability: the case for IPM as 'informed pest management'', *Pest Management Science*, vol. 65, pp. 1321-1328.

Castle, SJ 2001,' Differences between cotton and melon in host acceptance by *Bemisia tabaci*. In: *Proceedings of the Beltwide Cotton Conference*. National Cotton Council, Memphis, TN, pp. 1056–1059.

Castle, SJ, Palumbo, JC, Prabhaker, N, Horowitz, AR & Denholm, I 2010, 'Ecological determinants of *Bemisia tabaci* resistance to insecticides, in PA, Stansly & SE. Naranjo (eds.), *Bemisia: Bionomics and Management of a Global Pest*, Springer Science+Business Media, pp. 385-421.

Cervera, MT, Cabezas, JA, Simon, B, Martinez-Zapater, JM, Beitia, F & Cenis, JL. 2000, ' Genetic relationships among biotypes: Aleyrodidae) based on AFLP analysis', *Bulletin of Entomological Research, vol.*90, pp.391-396.

Chaudhuri, N, Deb, DC & Senapati, SK 2001,'Biology and fluctuation of white fly (*Bemisla tabacigenn*.) population on tomato as influenced by abiotic factors under terai region of west bengal', *Indian Journal of Agricultural Research*, vol. 35, no. 3, pp.155-160.

Choi, WI, Lee, EH, Choi, BR, Park, HM & Ahn, YJ 2003, ' Toxicity of plant essential oils to *Trialeurodes vaporariorum* Homoptera: Aleyrodidae', *Journal of Economical Entomology*, vol. 96, no.5, pp.1479-1484.

Chowdhury, NY, Islam, W & Khalequzzaman, M 2011,' Insecticidal activity of compounds from the leaves of *Vitex negundo* (Verbenaceae) against *Tribolium castaneum* (Coleoptera: Tenebrionidae)', *International Journal of Tropical Insect Science*, vol. 31, no. 3, pp. 174–181,

Christofoli, M, Costa, ECC, Bicalho, KU, de Cássia Domingues, V, Peixoto, MF, Alves, CCF, Araújo, WL & de Melo Cazal, C 2015, 'Insecticidal effect of nanoencapsulated essential oils from *Zanthoxylum rhoifolium* (Rutaceae) in *Bemisia tabaci* populations', *Industrial Crops and Products*, vol. 70, pp. 301-8.

Chu, CC, Natwick, ET, Henneberry, TJ & Lee, R 1998,' Effects of pyrethroid insecticides alone and in mixtures on *Bemisia argentifolii* (Homoptera: Aleyrodidae) and cotton, cauliflower and broccoli yields', *Journal of the Agricultural Association of China*, vol. 184, pp. 57-66.

Cis, J, Nowak, G & Kisiel, W 2006,' Antifeedant properties and chemotaxonomic implications of sesquiterpene lactones and syringin from *Rhaponticum pulchrum*', *Biochemical Systematics and Ecology*, Vol. 34, pp. 862-867.

Clemente, S, Mggiani, G, Broussalis, A. Martino, V & Ferraro, G 2003, 'Insecticidal effects of Lamiaceae species against stored products insects', *Boletín de Sanidad Vegetal Plagas*, vol. 29, pp.1-8.

Cock, MJW 1986,'Other control methods. In: Cock, M.J.W. (Ed.), *Bemisia tabaci - A Literature Survey*. CAB International Institute of Biological Control, Silkwood Park, UK, pp. 59–61.

Coppen, J.J.W. 1995. "*Flavour and Fragrances of Plant Origin*". *Non-wood Forest Products* I. Rome, Italy: Food and Agriculture Organisation (FAO).

Copping, LG & Menn, JJ 2000, 'Biopesticides: a review of their action, applications and efficacy', *Pest Management Science*, vol. 56, pp. 651-676.

Costa, HS & Brown, JK 1991, 'Variation in biological characteristics and esterase patterns among populations of *Bemisia tabaci*, and the association of one population with silverleaf symptom induction', *Entomologia Experimentalis Et Applicata*, vol. 61, pp. 211–219.

Costa, HS, Brown, JK, Sivasupramaniam, S & Bird, J 2003,' Regional distribution, insecticide resistance and reciprocal crosses between the A and B biotypes of *Bemisia tabaci', Insect Science and Its Application*, vol. 14, pp. 255–266.

Cruz-Estrada, A, Gamboa-Angulo, A, Borges-Argaez, R & Ruiz-Sanchez, E 2013,' Insecticidal effects of plant extracts on immature whitefly *Bemisia tabaci* Genn. (Hemiptera: Aleyroideae)', *Electronic Journal of Biotechnology*, Doi: 10.2225/vol16-issue1-fulltext-6.

Cuthbertson, AGS, Buxton, JH, Blackburn, LF, Mathers, JJ, Robinson, KA, Powell, ME, Fleming, DA & Bell, HA 2012,' Eradicating *Bemisia tabaci* Q biotype on poinsettia plants in the UK', *Crop Protection*, vol. 42, pp. 42-48.

David, WAL & Gardiner, BOC 1966, ' Mustard oil glucosides as feeding stimulants for *Pieris brassicae* larvae in a semi synthetic diet', *Entomologia Experimentalis* et *Applicata***,** vol. 9, pp. 247-255.

De Almeida Marques, M, Quintela, ED, Mascarin, GM, Fernandes, PM & Arthurs, SP 2014, 'Management of *Bemisia tabaci* biotype B with botanical and mineral oils', *Crop Protection*, vol. 66, pp. 127-32.

De Barro PJ 1995, '*Bemisia tabaci biotype B; A Review of its Biology, Distribution and Control',* 2nd edn, *CSIRO Division of Entomology, Technical Paper* No. 36. CSIRO, Canberra, Australia.

De Barro, PJ & Coombs, MT 2009, ' Post-release evaluation of *Eretmocerus hayati* Zolnerowich and Rose in Australia', *Bulletin of Entomological Research*, vol. 99, pp.193–206.

De Barro, PJ & Driver, F 1997,'Use of RAPD PCR to distinguish the B biotype from other biotypes of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae)', *Australian Journal of Entomology*, vol. 36, pp.149–152.

De Barro, PJ, Driver, F, Trueman, JWH & Curran, J 2000,'Phylogenetic relationships of world populations of *Bemisia tabaci* (Gennadius) using ribosomal ITS1', *Molecular Phylogenetics and Evolution,* vol. 16, no.1, pp.29-36.

De Barro, PJ, Liu, S, Boykin LM & Dinsdale, AB 2011,'*Bemisia tabaci*: a statement of species status', *Annual Review of Entomology,* vol. 56, pp.1-19.

Dehghani, M & Ahmadi, K 2013, 'Anti-oviposition and repellence activities of essential oils and aqueous extracts from five aromatic plants against greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae),'*Bulgarian Journal of Agricultural Science, Agricultural Academy,* vol. 19, no.4, pp. 691-696.

Dehghani, M, Ahmadi, K & Zohdi, H 2012, 'Evaluation of some plant extracts and conventional insecticides against *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) in greenhouse condition', *Munis Entomology & Zoology,* vol. 7, pp. 828-36.

Deletre, E, Chandre, F, Barkman, B, Menut, C, & Martin, T 2016, 'Naturally occurring bioactive compounds from four repellent essential oils against *Bemisia tabaci* whiteflies', *Pest Management Science*, vol. 72, pp. 179-89.

Denholm, I & Devine, G 2013,' Insecticides resistance', *Encyclopedia of Biodiversity (2nd. Ed,)*, pp. 298-307.

Denholm, I, Cahill, M, Dennehy, TJ & Horowitz, AR 1998, ' Challenges with managing insecticide resistance in agricultural pests, exemplified by whitefly *Bemisia tabaci*', *Philosophical Transactions of the Royal Society B: Biological Sciences,* vol. 353, pp.1757-1767.

Dimetry, NZ 2012,' Prospects of botanical pesticides for the future in integrated pest management programme (IPM) with special reference to neem uses in Egypt', *Archives of Phytopathology and Plant Protection*, vol. 45, no.10, pp. 1138-1161.

Drost, YC, Lenteren, JC van & Roermund, HJW van 1998,' Life-history parameters of different biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in relation to temperature and host plant: a selective review', *Bulletin of Entomological Research*, vol.88, pp.219-229.

Duffus, JE 1987, ' Whitefly transmission of plant viruses', *Current Topics in Vector Research,* vol. 4, pp. 73-91.

Eamsobhana, P, Yoolek, A, Kongkaew, W, Lerdthusnee, K, Khlaimanee, N, Parsartvit, A, Malainual, N & Yong, HS 2009,' Laboratory evaluation of aromatic essential oils from thirteen plant species as candidate repellents against *Leptotrombidium chiggers* (Acari: Trombiculidae), the vector of scrub typhus', *Experimental and Applied Acarology*, vol. 47, no. 3, pp. 257-62.

Elaine, B 2013,' Ethanolamines', *European Chemical News,* vol. 81, pp. 18.

Ellsworth, BC & Martinez-Carrillo, JL 2001,' IPM for *Bemisia tabaci*: a case study from North America', *Crop Protection*, vol. 20, no. 9, pp.853-869.

Ellsworth, PC 1999,' Whitefly management in Arizona cotton – status and needs. In: Dugger, P., Richter, D. (Eds.), *Proceedings Beltwide Cotton Conferences*. National Cotton Council, Memphis, TN, pp. 41–44.

Ellsworth, PC, Tronstad, R, Leser, J, Goodell, PB, Godfrey, LD, Henneberry, TJ, Hendrix, D, Brushwood, D, Naranjo, SE, Castle, S & Nichols, RL 1999, ' *Sticky cotton sources and solutions*. Univ. Arizona, Coop. Ext. Publ. No. AZ1156, IPM Series 13, 4pp.

El-Wakeil, NE 2013**, '**Botanical pesticides and their mode of action**',** *Gesunde Pflanzen*, vol. 65, pp. 125-149.

Erdogan, C, Moores, GD, Gurkan, MO, Gorman, KJ & Denholm, I 2008,' Insecticide resistance and biotype status of populations of the tobacco whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Turkey', *Crop Protection*, vol. 27, nos. 3-5, pp. 600-605.

Fanela, TLM, Baldin ELL, Pannuti, LER, Cruz, PL,' Crotti, AEM' Takeara, R, & Kato, MJ 2016, 'Lethal and inhibitory activities of plant-derived essential oils against *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) Biotype B in tomato*', Neotropical Entomology*, vol.45, pp. 201-210.

Farghaly, SF, Torkey, HM, Abou-Yousef, HM 2009, 'Natural extracts and their chemical constituents in relation to whitefly (*Bemisia tabaci*) and Aphid (*Aphis craccivora*)', *Australian Journal of Basic and Applied Sciences*, vol. 3, no.4, pp. 3217-3223.

Faria, M & Wraight, SP 2001, ' Biological control of *Bemisia tabaci* with fungi', *Crop Protection*, vol. 20, pp. 767–778.

Fazal, S, 1998,' Integrated pest management of cotton whitefly *Bemisia tabaci* and American bollworm [*Helicoverpa armigera*] on cotton', M.Sc. Thesis, Deptt. of Agri. Entomol., Univ. of Agric., Faisalabad.

Fenwick, GR, Heaney, RK & Mullin, WJ 1983, ' Glucosinolates and their breakdown products in food and food plants', *Critical Reviews in Food Science and Nutrition*, vol. 18, pp. 123-201.

Fernandes, GW 1990,' Hypersensitivity: a neglected plant resistance mechanism against insect herbivores', *Environmental Entomology*, vol. 19, pp.1173–1182.

Fernandez, E, Gra´valos, C, Haro, PJ, Cifuentes, D & Bielza, P 2009,' Insecticide resistance status of *Bemisia tabaci* Q-biotype in south-eastern Spain. *Pest Management Science*, vol. 65, pp. 885–891.

Fiume, L, Vettraino, M, Carnicelli, D, Arfilli, V, Di Stefano, G & Brigotti, M 2013, 'Galloflavin prevents the binding of lactate dehydrogenase A to single stranded DNA and inhibits RNA synthesis in cultured cells', *Biochemical and Biophysical Research Communications*, vol. 430, no. 2, pp. 466-9.

Flint, HM, Radin, JW, Parks, NJ & Reaves, LL 1995,' The effects of drip or furrow irrigation of cotton on *Bemisia argentifolii* (Homoptera: Aleyrodidae)', *Journal of Agricultural Entomology*, vol.12, pp. 25–32.

Flückiger, CR, Kristinsson H, Senn, R, Rindlisbacher, A Buholzer, H & Voss, G 1992,' CGA 215'944- a novel agent to control aphids and whiteflies control. In: *Brighton Crop Protection Conference – Pests and Diseases*, vol.1, pp. 43-50.

Frohlich, DR, Torres-Jerez, I, Bedford, ID, Markham, PG & Brown, JK 1999,'A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers', *Molecular Ecology*, vol. 8, pp.1683–91.

Gencsoylu, I, Horowitz, AR, Sezgin, F & Oncuer, C 2003,' Effect of drip and furrow irrigation methods on *Bemisia tabaci* populations in cotton fields', *Pytoparasitica*, vol. 31, no. 2, pp. 139-143.

Gennadius, P 1889,'Disease of tobacco plantations in the Trikonia. The aleyrodid of tobacco', *Ellenika Georgia*, vol. 5, pp.1-3.

Gerling, D & Mayer, RT 1996, *Bemisia: 1995. Taxonomy, Biology, Damage, Control and Management*, 1st edn, Andover, UK: Intercept.

Gerling, D & Naranjo, SE 1998,' The effect of insecticide treatments in cotton fields on the levels of parasitism of *Bemisia tabaci* (Gennadius) *sl*.', *Biological Control*, vol.12, pp. 33-41.

Gerling, D 1990, ' Natural enemies of whiteflies: predators and parasitoids', in D Gerling (ed.), *Whiteflies their Bionomics, Pest Status and Management*, Andover, UK: Intercept Ltd. pp. 147–186

Gerling, D, Alomar, Ò & Arnò, J 2001, 'Biological control of *Bemisia tabaci* using predators and parasitoids', *Crop Protection*, vol. 20, no. 9, pp. 779-99.

Giamoustaris, A & Mithen, R 1996, ' Genetics of aliphatic glucosinolates 4 Side-chain modification in *Brassica oleracea*', *Theoretical and Applied Genetics*, vol. 93, pp. 1006-1010.

Gill, RJ & Brown, JK 2010,'Systematics of *Bemisia* and *Bemisia* relatives: can molecular techniques solve the *Bemisia tabaci* complex conundrum-a taxonomist's viewpoint', in Stansly, PA & Narahjo, SE (eds), *Bemisia: Bionomics and Management of a Global Pest*. Springer, New York, USA. pp. 5-29.

Goettel, MS, Eilenberg, J & Glare, T 2005, 'Entomopathogenic fungi and their role in regulation of insect populations', *Comprehensive Molecular Insect Science*, Vol. 6, pp. 361-405.

Gonzalez-Coloma, A, Reina, M, Diaz, CE & Fraga, BM 2010,' 3.09 – Natural product-based biopesticides for insect control', *Comprehensive Natural Products II*, vol. 3, pp. 237-268.

Gonzalez-Zamora, JE, Leira, D, Bellido, MJ & Avilla, C 2004, 'Evaluation of the effect of different insecticides on the survival and capacity of *Eretmocerus mundus* Mercet to control *Bemisia tabaci* (Gennadius) populations', *Crop Protection*, vol. 23, no. 7, pp. 611-8.

Gullan, PJ & Cranston, PS 2000, *The insects: an outlines of entomology,* 2nd edn. Blackwell Science Ltd. pp. 269.

Gunning, RV, Byrne, FJ, Conde, BD, Connelly, MI, Hergstrom, K & Devonshire, AI 1995,' First report of B-biotype *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Australia', *Journal of the Australian Entomological Society*, vol. 34, pp. 116.

Gunning, RV, Byrne, FJ & Devonshire, AL 1997,'Electrophoretic analysis of non-B and G-biotype *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in Australia', *Australian Journal of Entomology*, vol. 36, pp.245-249.

Halkier, BA & Gershenzon, J 2006, 'Biology and biochemistry of glucosinolates', *Annual Review of Plant Biology*, vol. 57, pp. 303-33.

Han, E-J, Choi,B-R & Lee, J-H 2013,'Temperature-dependent development models of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) Q biotype on three host plants', *Journal of Asia-Pacific Entomology*, vol.16, no.1, pp.5–10.

Harrison, BD, Zhou, X, Otim-Nape, GW, Liu, Y & Robinson, DJ 1997, ' Role of a novel type of double infection in the geminivirusinduced epidemic of severe cassava mosaic in Uganda' , *Annals of Applied Biology,* vol. 131, pp. 437–448.

Hassan, E & Gökçe, A 2014, 'Production and Consumption of Biopesticides', in D Singh (ed.), *Advances in Plant Biopesticides*, Springer India, New Delhi, pp. 361-79.

He, Y, Zhao, J, Zheng, Y, Weng, Q, Biondi, A, Desneux, N & Wu, K 2013, 'Assessment of potential sublethal effects of various insecticides on key biological traits of the tobacco whitefly, *Bemisia tabaci*', *International Journal of Biological Sciences*, vol. 9, no. 3, pp. 246-55.

Helgason, E, Okstad, OA & Caugant, DA 2000,' *Bacillus anthracis*, *Bacillus cereus* and *Bacillus thuringiensis*: one species on the basis of genetic evidence', *Applied and Environmental Microbiology*, vol. 66, pp. 2627-30.

Hilje, L & Stansly, PA 2008,'Living ground covers for management of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) and tomato yellow mottle virus (ToYMoV) in Costa Rica, *Crop Protection*, vol. 27, pp. 10–16.

Hilje, L, Costa, HS & Stansly, PA 2001,'Cultural practices for managing *Bemisia tabaci* and associated viral diseases', *Crop Protection*, vol. 20, pp. 801-812.

Hooks, CRR, Valenzuelab, HR & Defrank, J 1998, 'Incidence of pests and arthropod natural enemies in zucchini grown with living mulches', *Agriculture, Ecosystems & Environment*, vol. 69, pp. 217-231.

Horowitz, AR, Kontsedalov, S, Khasdan, V& Ishaaya, I 2005,' Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance', *Archives of Insect Biochemistry and Physiology*, vol.58, pp. 216–225.

Hoy, MA 2013,' Chapter 14 – Genetic Modification of Pest and Beneficial Insects for Pest-Management Programs', in *Insect Molecular Genetics (3rd Ed): An Introduction to Principles and Applications*, pp. 661-736.

Ilias, A, Roditakis, E, Grispou, M, Nauen, R, Vontas, J & Tsagkarakou, A 2012,' Efficacy of ketoenols on insecticide resistant field populations of two-spotted spider mite *Tetranychus urticae* and sweet potato whitefly *Bemisia tabaci* from Greece', *Crop Protection*, vol. 42, pp. 305-311.

Inyang, EN, Butt, TM, Doughty, KJ, Todd, AD & Archer, S 1999 , ' The effect of isothiocyanates on the growth of the entomopathogenic fungus *Metarhizium anisopliae* and its infection of the mustared beetle', *Mycological Research*, vol. 103, pp. 974-980.

Iram, A Khan, J, Aslam, N, Ehsan-ul-Haq, J, Habib I, Irfan, M, Rasool, A, Mastoi, M I & Aslam, S 2014,'Efficacy of plant derived oils and extracts against white-fly, *Bemisia tabaci* (Gennadius) on sesame crop', *Pakistan Journal of Agricultural Research*, Vol. 27 no. 3, pp. 250-254.

Islam, MT & Ren, S 2007,'Development and reproduction of *Bemisia tabaci* on three tomato varieties', *Journal of Entomology*, vol. 4, no.3, pp.231-236.

Islam, MT, Qiu, B & Ren, S 2010,'Host preference and influence of the sweetpotato whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) on eggplant (*Solanum melongena* L.)', *Acta Agriculturae Scandinavica Section B-Soil and Plant Science*, vol. 60, pp.320-325.

Isman, MB 2000,'Plant essential oils for pest and disease management', *Crop Protection*, vol.19, pp.603-608.

Isman, MB 2006,' Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world', *Annual Review of Entomology*, Vol. 51, pp. 45-66.

Isman, MB, Miresmailli, S & Machial, C 2011,' Commercial opportunities for pesticides based on essential oils in agriculture, industry and consumer products', *Phytochemistry Reviews*, vol. 10, pp. 197-204.

Jafarbeigi, F, Samih, MA, Zarabi, M, Esmaeily, S & Izadi, H 2011,' Study on susceptibility of *Bemisia tabaci* (Genn.)(Biotype A) to *Calotropis procera* and *Fumaria parviflora* plant extracts in control conditions. 471 pp. In *Global Conference on Entomology*, March 5-9, 2011, Chiang Mai, Thailand. Century Foundation, India.

Jauset, AM, Sarasuua, MJ, Avilla, J & Albajes, R 2000, 'Effect of nitrogen fertilization level applied to tomato on the greenhouse whitefly', *Crop Protection*, vol. 19, pp. 255-261.

Javed, MA & Matthews, GA 2002, 'Bioresidual and integrated pest management status of a biorational agent and a novel insecticide against whitefly and its key parasitoids', *International Journal of Pest Management*, vol. 48, no. 1, pp. 13-7.

Jeschke, P, Nauen, R, Schindler, M & Elbert, A 2011,' Overview of the status and global strategy for neonicotinoids', *Journal of Agricultural and Food Chemistry*, vol. 59, pp. 2897-2908.

Jones, DR 2003, 'Plant viruses transmitted by whiteflies' , *European Journal of Plant Pathology*, vol. 109, no.3, pp. 195-219.

Kalawate, A & Dethe, MD 2012,' Bioefficacy study of biorational insecticide on brinjal', *Journal of Biopesticides*, vol.5, no.1, pp. 75-80.

Karut, K & Naranjo, SE 2009, ' Mortality factors affecting *Bemisia tabaci* populations on cotton the Çukurova plain, Turkey', *Journal of Applied Entomology*, vol. 133, pp. 367–374.

Kayser, H, Kaufmann, L & Schurmann, F 1994,' Pymetrozine (CGA 215'944): a novel compound for aphid and whitefly control. In: An overview of its mode of action', *Proceedings of 1994 Brighton crop protection conference – pests and diseases*, vol.2, pp. 737-742.

Khachatourians, GG 2009,' Insecticides, Microbial', *Encyclopedia of Microbiology (3rd ed.),* pp. 95-109.

Khan, MR, Ghani, IA, Khan, MR, Abdul Ghaffar & Tamkeen, A 2011,'Host plant selection and oviposition behaviour of whitefly *Bemisia tabaci* (Gennadius) in a mono and simulated polyculture crop habitat', *African Journal of Biotechnology*, vol.10, no.8, pp.1467-1472.

Kim, S-I, Chae, S-H, Youn, H-S, Yeon, S-H & Ahn, Y-J 2011, 'Contact and fumigant toxicity of plant essential oils and efficacy of spray formulations containing the oils against B- and Q-biotypes of *Bemisia tabaci*', *Pest Management Science*, vol. 67, no. 9, pp. 1093-9.

Kontsedalov, S, Gottlieb, Y, Ishaaya, I, Nauen, R, Horowitz, AR & Ghanim, M 2009,' Toxicity of spiromesifen to the developmental stages of *Bemisia tabaci* biotype B', *Pest Management Science*., vol. 65, pp. 5-13.

Korolev, N & Gindin G 1999,' Vegetative compatibility in the entomopathogen *Verticillium lecanii*', *Mycological Research*, vol. 103, no.7, pp. 833-840.

Koul, O & Wahab, S 2004,' *Neem: Today and in the New Millennium*, Kluwer Academic Publishers, Dordreeht.

Krehic, M & Avenel-Audran, M 2009, 'Allergic contact dermatitis from decyl glucoside in an antiseptic lotion', *Contact Dermatitis*, vol. 61, no. 6, pp. 349-50.

Kulkarni, J, Kapse, N & Kulkarni, DK 2009, 'Plant-based pesticides for control of *Helicoverpa armigera* on *Cucumis sativus*' , *Asian Agri-history*, vol.13, no.4, pp. 327-332.

Kumar, P, Poehling, HM & Borgemeister, C 2005, 'Effects of different application methods of azadirachtin against sweetpotato whitefly *Bemisia tabaci* Gennadius (Hom., Aleyrodidae) on tomato plants', *Journal of Applied Entomology*, vol. 129, no. 9-10, pp. 489-97.

Kumar, P, Whitten, M, Thoeming, G, Borgemeister, C & Poehling, HM 2008, 'Effects of bio-pesticides on *Eretmocerus warrae* (Hym., Aphelinidae), a parasitoid of *Bemisia tabaci* (Hom., Aleyrodidae) ', *Journal of Applied Entomology*, vol. 132, pp. 605–613.

Kumarasinghe, NC, Salim, N & Wijayarathne, W 2009,'Identification and biology of two whitefly species on Cassava in Sri Lanka', *Journal of Plant Protection Research*, vol. 49, no.4, pp.373-377.

Lahey, Z & Stansly, P 2015, 'An Updated List of Parasitoid Hymenoptera Reared from the *Bemisia tabaci* Species Complex (Hemiptera: Aleyrodidae)', *Florida Entomologist*, vol. 98, no. 2, pp. 456-63.

Lazzeri, L, Curto, G, Leoni, O & Dallavalle, E 2004, ' Effects of glucosinolates and their enzymatic hydrolysis products via myrosinase on the root-knot nematode *Meloidogyne incognita* (Kofoid et White) Chitw', *Journal of Agricultural and Food Chemistry*, vol. 52, pp. 6703- 6707.

Lee, D-H, Nyrop, JP& Sanderson, JP 2011, 'Avoidance of natural enemies by adult whiteflies, *Bemisia argentifolii*, and effects on host plant choice', *Biological Control*, vol. 58, pp. 302–309.

Legg, JP 1999, ' Emergence, spread and strategies for controlling the pandemic of cassava mosaic virus disease in east and central Africa', *Crop Protection*, vol. 18, pp. 627–637.

Li, JJ, Tang, QB, Bai, RE, Li, XM, Jiang, JW, Zhai Q & Yan FM 2013,' Comparative morphology and morphometry of six biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from China', *Journal of Integrative Agriculture*, vol. 12, no. 5, pp. 846-852.

Li, S, Vinson, B & Gerling, D 1989,'Courtship and mating behavior of *Bemisia tabaci* (Homoptera: Aleyrodidae)', *Environmental Entomology*, vol.18, pp.800-806.

Lima, LHC, Moretzohn, MC & Oliveira, MRV 2000, ' Survey of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotypes in Brazil using RAPD markers', *Genetics and Molecular Biology,* vol. 23, pp. 1–5.

Liu, S-S, Colvin, J & De Barro, PJ 2012,' Species concepts as applied to the whitefly *Bemisia tabaci* systematics: how many species are there?', *Journal of Integrative Agriculture*, vol. 11, no.2, pp.176-186.

Liu, T-X 2004, 'Toxicity and efficacy of spiromesifen, a tetronic acid insecticide, against sweetpotato whitefly (homoptera: aleyrodidae) on melons and collards', *Crop Protection*, vol. 23, no. 6, pp. 505-13.

Liu, TX 2007, 'Life history of *Eretmocerus melanoscutus* (Hymenoptera: Aphelinidae) parasitizing nymphs of *Bemisia tabaci* Biotype B (Homoptera: Aleyrodidae)', *Biological Control*, vol. 42, no. 1, pp. 77-85.

Liu, TX & Stansly, PA 1995,' Toxicity of biorational insecticides to *Bemisia argentifolii* (Homoptera: Aleyrodidae) on tomato leaves', *Journal of Economic Entomology*, vol.88, pp. 564-568.

Liu, TX & Stansly, PA 2000,' Insecticidal activity of surfactants and oils against silverleaf whitefly (*Bemisia argentifolii*) nymphs (Homoptera: Aleyrodidae) on collards and tomato', *Pest Management Science*, vol. 56, no. 10, pp. 861-866.

Liu, TX, Stansly, PA & Chortyk, OT 1996,' Insecticidal activity of natural and synthetic sugar esters against *Bemisia argentifolii* (Homoptera: Aleyrodidae)', *Journal of Economic Entomology*, vol. 89, pp. 1233-1239.

Liu, XC, Hu, JF, Zhou, L, & Liu, ZL 2014, ' Evaluation of fumigant toxicity of essential oils of Chinese medicinal herbs against *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) *', Journal of Entomology and Zoology Studies*, vol. 2, pp. 164-169.

López, SN & Andorno, AV 2009,' Evaluation of the local population of *Eretmocerus mundus* (Hymenoptera: Aphelinidae) for biological control of *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) in greenhouse peppers in Argentina', *Biological Control*, vol. 50, no.3, pp. 317-323.

Lowery, DT & Isman, MB 1994, 'Insect growth regulating effects of neem extract and azadirachtin on aphids', *Entomologia Experimentalis Et Applicata*, vol. 72, no. 1, pp. 77-84.

Luo, C, Jones, CM, Devine, G, Zhang, F, Denholm, I & Gorman, K 2010,' Insecticide resistance in *Bemisia tabaci* biotype Q (Hemiptera: Aleyrodidae) from China', *Crop Protection*, vol.29, pp. 429–434.

Ma, D, Gorman, K, Devine, G, Luo, W & Denholm, I 2007,' The biotype and insecticide-resistance status of whiteflies, *Bemisia tabaci* (Hemiptera: Aleyrodidae), invading cropping systems in Xinjiang Uygur Autonomous Region, northwestern China', *Crop Protection*, vol. 26, no.4, pp. 612-617.

Ma, DY, Li, XC, Dennehy, TJ, Lei, CL & Wang, M 2009,'Utility of mtCO1 polymerase chain reaction-restriction fragment length polymorphism in differentiating between Q and B whitefly *Bemisia tabaci* biotypes', *Insect Science and Its Application*, vol.16, pp.107–14.

Manici, LM, Lazzeri L & Palmieri, S 1997, ' In vitro Fungitoxic activity of some glucosinolates and their enzyme-derived products toward plant pathogenic fungi. *Journal of Agricultural and Food Chemistry*', vol. 45, pp. 2768-2775.

Mansour, SAA, Mohamad Roff, MN, Saada, KA, Abuzida, I & Idris, AB 2012,'Responses of whitefly, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae) population on tomato *Lycopersicon*

esculentnm mixed with other crops under glasshouse conditions', *APCBEE Procedia*, vol. 4, pp.48–52.

Mansour, SA, El-Sharkawy, AZ & Abdel-Hamid, NA 2015, 'Toxicity of essential plant oils, in comparison with conventional insecticides, against the desert locust, *Schistocerca gregaria* (Forskål)', *Industrial Crops and Products*, vol. 63, pp. 92-9.

Martínez, MRM, Martínez, WAM & Mera, CAV 2009,'*Bemisia tabaci* biotype B in bean: Life history parameters and absence of host-parasitoid interaction with *Amitus fuscipennis*', *Acta Agronómica,* vol.58, no.4, pp.251-259.

McDonnell, G & Russell, AD 1999,' Antiseptics and disinfectants: activity, action, and resistance', *Clinical Microbiology Reviews*, vol.12, pp.147–179.

McKenzie, CL, Weathersbee, AA, Hunter, WB & Puterka, GJ 2004, 'Sucrose Octanoate Toxicity to Brown Citrus Aphid (Homoptera: Aphididae) and the parasitoid *Lysiphlebus testaceipes* (Hymenoptera: Aphidiidae)', *Journal of Economic Entomology*, vol. 97, no. 4, pp. 1233-8.

McKenzie, CL, Weathersbee, AA & Puterka, GJ 2005,' Toxicity of sucrose octanoate to egg, nymphal, and adult *Bemisia tabaci* (Hemiptera: Aleyrodidae) using a novel plant-based bioassay', *Journal of Economic Entomology*, vol.98, no.4, pp.1242-7.

Micon national 2005, *Chemwatch MSDS 5129-31*, viewed 22 April 22, 2016, http://www.miconnational.com.au/pdfs/MSDS%20%20JS%20Hayes_Trix%20Dishwashing(82240).pdf.

Misra, CS & Lamba, SK 1929, 'The cotton whitefly (*Bemisia gossypiperda* n. sp.)', *Bulletin of the Agricultural Research Institute*, vol. 196, pp. 1–7.

Mohamed, MA 2012, 'Impact of planting dates, spaces and varieties on infestation of cucumber plants with whitefly, *Bemisia tabaci* (Genn.)', *The Journal of Basic and Applied Zoology*, vol. 65, pp. 7–20.

Moore, AD, Sequeira, RV & Woodger, TA 2004,' Susceptibility of crop plants to *Bemisia tabaci* (Gennadius) B-biotype (Hemiptera: Aleyrodidae) in central Queensland, Australia', *Australian Entomologist*, vol. 31, pp. 69–74.

Moriones, E & Navas-Castillo, J 2000, ' Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide' , *Virus Research*, vol. 71, pp.123-134.

Morales, FJ 2006,' History and Current Distribution of Begomoviruses in Latin America', *Advances in Virus Research*, Vol. 67, pp. 127-162.

Mound LA & Halsey SH 1978, *Whitefly of the World. A Systematic Catalogue of the Aleyrodidae (Homoptera) with Host Plant and Natural Enemy Data,* Chichester, UK:Wiley.

Nakatsu, T, Lupo, AT, Chinn, JW, & Kang, RKL 2000**,'** Biological activity of essential oils and their constituents**',** Studies *in Natural* Products Chemistry**,** ***vol.*** 21, pp. 571-631.

Naranjo, SE & Ellsworth PC 1999, 'Mortality factors affecting whitefly populations in Arizona cotton: life table analysis. *In* J.C. Silvertooth [ed.], Cotton, A College of Agriculture Report. University of Arizona, College of Agriculture, Tucson, AZ. Viewed 30 September 2015 < http://extension.arizona.edu/sites/extension.arizona.edu/files/pubs/az11237j.pdf>.

Naranjo, SE & Ellsworth, PC 2009a,' The contribution of conservation biological control to integrated control of *Bemisia tabaci* in cotton', *Biological Control*, vol. 51, no.3, pp. 458-470.

Naranjo, SE & Ellsworth, PC 2009b, 'Fifty years of the integrated control concept: Moving the model and implementation forward in Arizona', *Pest Management Science*, vol. 65, no. 12, pp. 1267-1286.

Naranjo, SE, Ellsworth, PC & Haglera, JR 2004, 'Conservation of natural enemies in cotton: role of insect growth regulators in management of *Bemisia tabaci*', *Biological Control*, vol. 30, pp. 52-72.

Naranjo, SE, Ellsworth, PC, Chu, CC, Henneberry, TJ, Riley, DG, Watson, TF & Nichols, RL 1998,' Action thresholds for the management of *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton', *Journal Economic Entomology*, vol. 90, pp. 1415-1426.

Nauen, R, Schnorbach, HJ & Elbert, A 2005,' The biological profile of spirmesifen (Oberon) - a new tetronic acid insecticide/ acaricide', *Pflanzenschutz-Nachrichten Bayer*, vol. 58, pp. 417-440.

Naveed, M, Salam, A, Saleem, MA & Sayyed, AH 2008,' Effect of foliar applications of some insecticides on *Bemisia tabaci*, predators and parasitoids: Implications in its management in Pakistan', *Phytoparasitica*, vol. 36, no. 4, pp. 377-387.

Neal, JW, Buta, JG, Pittarelli, GW, Lusby, WR & Benz, JA 1994,' Novel sucrose esters from *Nicotiana gossei*: effective biorationals against selected horticultural insect pests', *Journal of Economic Entomology*, vol. 87, pp. 1600-1607.

Ndomba, O 2007,' Surveillance for whiteflies on greenhouse roses and chrysanthemums in northern Tanzania', *EPPO Bulletin,* vol. 37, pp.407-411.

Nombela, G & Muniz, M 2010, 'Host Plant Resistance for the Management of *Bemisia tabaci*: A Multi-crop Survey with Emphasis on Tomato'. in PA Stansly & SE Naranjo (eds.), *Bemisia: Bionomics and Management of a Global Pest*, Springer Science+Business Media, pp. 357-383.

Nombela, G, Beitia, F & Muñiz, M 2000, ' Variation in tomato host response to *Bemisia tabaci* (Hemiptera: Aleyrodidae) in relation to acylsugar content and presence of the nematode and potato aphid resistance gene Mi', *Bulletin Entomological Research*, vol. 90, pp. 161–167.

Nombela, G, Garzo, EI, Duque, M, Fereres, A & Muñiz, M 2004, 'Resistance to *Bemisia tabaci* is induced in tomato plants after aphid infestation', *Compendium of the 2nd European Whitefly Symposium. IWSN-Institute for Adriatic Crops & Karst Reclamation*. Cavtat, pp. 63–64.

Nomikou, M, Janssen, A, Schraag, R & Sabelis, MW 2002,' Phytoseiid predators suppress populations of *Bemisia tabaci* on cucumber plants with alternative food', *Experimental and Applied Acarology*, vol. 27, pp. 57–68.

Oliveira, MRV, Henneberry, TJ & Anderson, P 2001, ' History, current status and collaborative research projects for *Bemisia tabaci*' , *Crop Protection*, vol. 20, pp. 709-723.

Oriani, MA de G, Vendramim, DJ & Vasconcelos, C. J 2011,'Biology of *Bemisia tabaci* (Genn.) B biotype (Hemiptera, Aleyrodidae) on tomato genotypes', *Scientia Agricola (Piracicaba, Brazil)*, vol. 68, no.1, pp.37-41.

Paes, JL, Faroni, LRDA, Dhingra, OD, Cecon, PR & Silva, TA 2012, 'Insecticidal fumigant action of mustard essential oil against *Sitophilus zeamais* in maize grains', *Crop Protection*, vol. 34, no. 0, pp. 56-8.

Palumbo, JC, Horowitz, AR & Prabhaker, N 2001,' Insecticidal control and resistance management for *Bemisia tabaci'*, *Crop Protection*, vol. 20, pp. 739-765.

Parmar, E & Burridge, A 2004,‘ Europe chemical profile: ethanolamines’, ICIS Chemical Business, viewed on 5 November 2014,< http://www.icis.com/resources/news/2012/04/23/9552129/europe-chemical-profile-ethanolamines/>.

Pavela, R & Herda, G 2007, 'Repellent effects of pongam oil on settlement and oviposition of the common greenhouse whitefly *Trialeurodes vaporariorum* on chrysanthemum', *Insect Science*, vol. 14, no. 3, pp. 219-24.

Pener, MP & Dhadialla, TS 2012,' An overview of insect growth disruptors; applied aspects', *Advances in Insect Physiology*, vol. 43, pp. 1-162.

Perkins, HH 1983, ' Identification and processing of honeydew contaminated cottons', *Textile Research Journal*, pp.508-12.

Perring, TM 2001,' The *Bemisia tabaci* species complex', *Crop Protection*, vol. 20, pp.725-737.

Pickett, CH, Keaveny, D & Rose, M 2013, 'Spread and non-target effects of *Eretmocerus mundus* imported into California for control of *Bemisia tabaci*: 2002–2011', *Biological Control*, Vol. 65, no.1, pp. 6-13.

Pimentel, D 2005,' Environmental and economic costs of the application of pesticides primarily in the United States', *Environment, Development and Sustainability*, vol. 7, pp. 229-252.

Pinheiro, PV, Quintela, ED, Oliveira, JP & Seraphin, JC 2009,' Toxicity of neem oil to *Bemisia tabaci* nymphs reared on dry bean', *Pesquisa Agropecuária Brasileira,* vol. 44, no.4, pp.354-360.

Pinto-Zevallos, D & Vanninen I 2013,' Yellow sticky traps for decision-making in whitefly management: What has been achieved?', *Crop Protection*, vol. 47, pp.74-84.

Pittarelli, GW, Buta, JG, Neal, JW, Jr.; Lusby, WR, & Waters, RM 1993, *'Biological pesticide derived from nicotiana plants*', Erteilung US. Patent No. 5260281

Pope, T, Kissen, R, Grant, M, Pickett, J, Rossiter, J & Powell, G 2008, ' Comparative innate responses of the aphid parasitoid *Diaeretiella rapae* to alkenyl glucosinolate derived isothiocyanates, nitriles, and epithionitriles', *Journal of Chemical Ecology*, vol. 34, pp.1302-1310.

Prabhaker, N, Morse, JG, Castle, SJ, Naranjo, SE, Henneberry, TJ & Toscano, NC 2007, 'Toxicity of seven foliar insecticides to four insect parasitoids attacking citrus and cotton pests', *Journal of Economic Entomology*, vol. 100, no. 4, pp. 1053-61.

Püntener W., 1981 Manual for field trials in plant protection second edition. Agricultural Division, Ciba-Geigy Limited.

Puri, SN, Bhosle, BB, Ilyas, M, Butler Jr, GD & Henneberry, TJ 1994, 'Detergents and plant-derived oils for control of the sweetpotato whitefly on cotton', *Crop Protection*, vol. 13, no. 1, pp. 45-8.

Puterka. GJ, Farone, W, Palmer, T, & Barrington, A 2003 , 'Structure-function re- lationships affecting the insecticidal and miticidal activity of sugar esters', *Journal of Economy Entomology*, vol. 96, pp. 636-44.

Quaintance, AL 1900,'Contribution towards amonograph of the American Aleurodidae'. *US Department of Agriculture. Technical Series. Bureau of Entomology*, vol. 8, pp.9-64.

Raguraman, S 2009,' Neem and environment: integration of neem with bioagents in insect pest management', *Madras Agricultural Journal*, vol. 96, pp. 277-282.

Regnault-Roger, C 1997,' The potential of botanical essential oils for insect pest control', *Integrated Pest Management Reviews*, vol. 2, pp. 25-34.

Regnault-Roger, C, Vincent, C, & Arnason, JT 2012,'Essential oils in insect control: low-risk products in a high-stakes world*', Annual Review* of *Entomology,* vol.57, pp.405-424.

Rosell, RC, Bedford, ID, Frohlich, DR, Gill, RJ, Brown, JK, & Markham, PG 1997,'Analysis of morphological variation in distinct populations of *Bemisia tabaci* (Homoptera: Aleyrodidae)', *Annals of the Entomological Society of America*, vol. 90, pp.575–89.

Ruder, FJ, Guyer, W, Benson, JA & Kayser, H 1991,' The thiourea insecticide/ acaricide diafenthiuron has a novel mode of action: inhibition of mitochondrial respiration by its carbdiimide product', *Pesticide Biochemistry and Physiology*, vol. 41, pp. 207-219.

Saad, NY, Muller, CD, & Lobstein, A 2013,' Major bioactivities and mechanism of action of essential oils and their components*', Flavour and Fragrance Journa*l, vol. 28, pp. 269-279.

Saguez, J, Attoumbre, J,Giordanengo,P & Baltora-Rosset, S 2013,' Biological activities of lignans and neolignans on the aphid *Myzus persicae* (Sulzer)', *Arthropod-Plant Interactions*, vol. 7, pp. 225–233.

Salas, J & Mandoza, O 1995,' Biology of the sweetpotato whitefly (Homoptera: Aleyrodidae) on tomato', *Florida Entomologist*, vol. 78, no.1, pp. 154-160.

Salimon, J, Salih, N & Yousif, E 2012, 'Industrial development and applications of plant oils and their biobased oleochemicals', *Arabian Journal of Chemistry*, vol. 5, no. 2, pp. 135-45.

Santos, JC, Faroni, LRA, Sousa, AH & Guedes, RNC 2011, 'Fumigant toxicity of allyl isothiocyanate to populations of the red flour beetle *Tribolium castaneum*', *Journal of Stored Products Research*, vol. 47, no. 3, pp. 238-43.

Schilick-Souza, EC, Baldin, ELL & Lourencao, AL 2011, 'Variation in the host preferences and responses of *Ascia monuste orseis* Godart (Lepidoptera: Pieridae) to cultivars of collard greens *Brassica oleracea* (L.) var. *acephala*', *Journal of Pest Science*, vol. 84, pp. 429–436.

Schmutterer, H 1990, 'Properties and Potential of Natural Pesticides from the Neem Tree, *Azadirachta Indica'*, *Annual Review of Entomology*, vol. 35, no. 1, pp. 271-97.

Schmutterer, H. 1992 Einfluß von Azadirachtin, einer azadirachtinfreien Fraktion eines alkoholischen Niemsamenextraktes und von formulierten Extrakten auf Verpuppung, Schlupf und Imagines der Kohlweißlingsbrackwespe *Apanteles glomeratus (L.)* (Hym., Braconidae), *Journal of Applied Entomology*, *vol.*113, pp. 79–87.

Schuster, DJ 2005,'Scouting for insects, use of thresholds and conservation of beneficial insects on tomatoes', Document ENY-685. Entomology department, Florida Cooperative Service, Institute of Food and Agrigultural Sciences, University of Florida (US.).

Sequeira, RV & Naranjo, SE 2008,' Sampling and management of *Bemisia tabaci* (Genn.) in Australian cotton', *Crop Protection*, vol. 27, no.9, pp. 1262-1268.

Sertkaya, E, Kaya, K & Soylu, S 2010, 'Acaricidal activities of the essential oils from several medicinal plants against the carmine spider mite (*Tetranychus cinnabarinus* Boisd.) (*Acarina: Tetranychidae*)', *Industrial Crops and Products*, vol. 31, no. 1, pp. 107-12.

Setiawati, W, Udiarto, BK & Gunaeni, N 2009,'Preference and infestation pattern of *Bemisia tabaci* (genn) on some tomato varieties and its effect on Gemini virus infestation', *Indonesian Journal of Agriculture.* Vol. 2, no.1, pp.57-64.

Severson, RF, Jackson, DM, Johnson, A.W, Sisson, VA & Stephenson, MG 1991, 'Ovipositional behavior of tobacco budworm and tobacco hornworm', in: P.A. Hedin (ed.), *Naturally Occurring Pest Bioregulators*, *ACS Symposium Series*, Vol. 449, pp. 264-277.

Sharaf, NS, Al-Musa, AM & Batta, Y 1985,'Effect of different host plants on population development of the sweetpotato whitefly (*Bemisia tabaci* Genn., Homoptera: Aleyrodidae)'. *Dirasat* XII, No. 6.

Simmonds, MSJ, Manlove, JD, Blaney, WM & Khambay, BPS 2002, 'Effects of selected botanical insecticides on the behaviour and mortality of the glasshouse whitefly *Trialeurodes vaporariorum* and the parasitoid *Encarsia formosa*', *Entomologia Experimentalis Et Applicata*, vol. 102, no. 1, pp. 39-47.

Simmons, AM & Shaaban, A-R 2011, 'Populations of predators and parasitoids of *Bemisia tabaci* (Hemiptera: Aleyrodidae) after the application of eight biorational insecticides in vegetable crops', *Pest Management Science*, vol. 67, no. 8, pp. 1023-8.

Singh, J, Sohi, AS, Brar, DS, Denholm, I, Russel, D & Briddon, R 1999, ' Management of cotton leaf curl viral disease in India. In: Proceeding of the ICAC-CCRI Regional Consultation, Insecticide Resistance Management in Cotton. *Central Cotton Research Institute, Multan, Pakistan*, pp. 277–278.

Smith, PG 1944, 'Embryo culture of a tomato species hybrid', *Proceedings American Society of Horticultural Science*, vol. 44, pp. 413–416.

Snowdon, R, Lühs, W & Friedt, W 2007, 'Oilseed rape', in: C. Kole (ed.), Genome mapping and molecular breeding in plants, vol. 2: Oilseeds, Springer, Berlin Heidelberg, pp. 55-114.

Sohrabi, F, Shishehbor, P, Saber, M & Mosaddegh, MS 2013, 'Lethal and sublethal effects of imidacloprid and buprofezin on the sweetpotato whitefly parasitoid *Eretmocerus mundus* (Hymenoptera: Aphelinidae)', *Crop Protection*, vol. 45, pp. 98-103.

Spectrum chemical 2006, *Material safety data sheet*, viewed 22 April 2016, https://www.spectrumchemical.com/MSDS/M4210.pdf.

Sporleder, M & Lacey, LA 2012, Biopesticides, in A Alykhin, C Vincent & P Giordanengo (eds) *Insect pests of potato*, Elsever Inc. pp. 463-497.

Stansly, PA, Liu, TX & Vavrina, CS 1998, 'Response of *Bemisia argentifolii* (Homoptera: Aleyrodidae) to imidacloprid under greenhouse, field and laboratory conditions', *Journal of Economic Entomology*, vol. 91, pp. 686–692.

Stansly, PA & Natwick, Et 2010,' Integrated systems for managing *Bemisia tabaci* in protected and open field agriculture', in PA, Stansly & SE. Naranjo (eds.), *Bemisia: Bionomics and Management of a Global Pest*, Springer Science+Business Media, pp.467-497.

Stansly, PA, Sanchez, PA, Rodriguez, JM, Canizares, F, Nieto, A, Lopez-Leyva, MJ, Fajardo, M, Suarez, V & Urbaneja, A 2004,' Prospects for biological control of *Bemisia tabaci* (Homoptera, Aleyrodidae) in greenhouse tomatoes of southern Spain', *Crop Protection*, vol. 23, pp. 701-712.

Stansly, PA, Calvo, J & Urbaneja, A 2005,'Release rates for control of *Bemisia tabaci* (Homoptera: Aleyrodidae) biotype "Q" with *Eretmocerus mundus* (Hymenoptera: Aphelinidae) in greenhouse tomato and pepper', *Biological Control*, vol. 35, no.2, pp.124-133.

Steinemann, A, Stamm, E & Frei, B 1990,' Cemodynamics in research and development of new plant protection agents', *Pesticide Outlook*, vol. 3, no. 1, pp. 3-7.

Stern, VM, Smith, RF, den Bosch, RV & Hagen, KS 1959,' The integrated control concept', *Hilgardia*, vol.29, pp. 81–101.

Stott, WT & Kleinert, KM 2008,' Effect of diisopropanolamine upon choline uptake and phospholipid synthesis in Chinese hamster ovary cells', *Food and Chemical Toxicology*, Vol. 46, no. 2, pp. 761–766.

Stout, MJ, Zehnder, GW & Baur, ME 2002, ' Potential for the use of elicitors of plant resistance in arthropod management programs', *Archives of Insect Biochemistry and Physiology, vol.* 51, pp. 222–235.

Sudakin, DL 2003,' Biopesticides', *Toxicological reviews*, vol. 22, no.2, pp. 83-90.

Tabashnik, BE & Carriere, Y 2007,' Field evaluation of resistance to pyriproxyfen in *Bemisia tabaci* (B biotype)', *Journal of Economic Entomology*, vol. 100, pp. 1650-1656.

Thompson, WMO, 2011, 'Introduction: Whiteflies, Geminiviruses and Recent Events', in WMO Thompson (Ed.) *The Whitefly, Bemisia tabaci (Homoptera: Aleyrodidae) Interaction with Geminivirus-Infected Host Plants: Bemisia tabaci, Host Plants and Geminiviruses ,* Springer, pp. 1-13.

Tomizawa, M & Casida, JE 2005,' Neonicotinoid insecticide toxicology: mechanisms of selective action', *The Annual Review of Pharmacology and Toxicology*, vol. 45, pp.247–68.

Tomizawa, M & Yamamoto, I 1993,' Structure–activity relationships of nicotinoids and imidacloprid analogs', *Journal of Pesticide Science*, vol. 18, pp. 91–98.

Urbaneja, A & Stansly, PA 2004,'Host suitability of different instars of the whitefly *Bemisia tabaci* biotype "Q" for *Eretmoserus mundus*', *BioControl*, vol. 49, no.2, pp.153-161.

Urbaneja, A, Sánchez, E & Stansly, PA 2007, ' Life history of *Eretmocerus mundus* Mercet (Hym.: Aphelinidae), a parasitoid of *Bemisia tabaci* Gennadius (Hom: Aleyrodidae), on tomato and sweet pepper', *BioControl*, vol. 52, pp. 25–39.

Uzama, D, Orishadipe, AT & Danhalilu, RL 2016, ' Phytochemical, nutritional and antimicrobial evaluations of the aqueous extract of *Brassica nigra* (Brassicaceae) seeds', *American Journal of Applied Chemistry*, vol. 4, pp. 161-163.

Valverde, RA, Jeonggu Sim, J & Lotrakul, P 2004, ' Whitefly transmission of sweet potato viruses', *Virus Research*, vol.100, pp.123–128.

van Lenteren, JC & Noldus, LPJ 1990,'Whitefly-plant relationship: behavioral and ecological aspects', in D Gerling (Ed.) *Whiteflies: their Bionomics, Pest Status and Management*,), Intercept, Andover, Hants, UK, pp. 47-89.

Vaughn, SF & Boydston, RA 1997, ' Volatile allelochemicals released by crucifer green manures', *Journal of Chemical Ecology*, vol. 23, pp. 2107-2116.

Villanueva-Jimenez, JA, Schellhorn, NA & De Barro, PJ 2012,' Comparison between two species of *Eretmocerus* (Hymenoptera: Aphelinidae): Reproductive performance is one explanation for more effective control in the field', *Biological Control*, vol. 63, no.3, pp. 333-338.

Villar, A, G!omez, E, Morales, F & Anderson, P 1998, 'Effect of legal measures to control *Bemisia tabaci* and geminiviruses in the Valley of Azua. Report from the National Integrated Pest Management Program, Santo Domingo, Dominican Republic, 16pp.

Wang, K & Tsai, JH 1996,' Temperature effects on development and reproduction of silverleaf whitefly (Homoptera, Aleyrodidae)', *Annals of the Entomological Society of America*, vol. 89, pp.375-384.

Wei, D-Z, Zou, P, Tu, M-B & Zheng, H 2002, 'Enzymatic synthesis of ethyl glucoside lactate in non-aqueous system', *Journal of Molecular Catalysis B: Enzymatic*, vol. 18, no. 4–6, pp. 273-8.

Wilson, AE, Bergaentzlé, M, Bindler, F, Marchioni, E, Lintz, A & Ennahar, S 2013, 'In vitro efficacies of various isothiocyanates from cruciferous vegetables as antimicrobial agents against foodborne pathogens and spoilage bacteria', *Food Control*, vol. 30, no. 1, pp. 318-24.

Wolfson, JL 1982, ' Developmental responses of *Pieris rapae* and *Spodoptera eridania* to environmentally induced variation in *Brassica nigra*', *Environmental Entomology*, vol, 11, pp, 207- 213.

Wraight, SP, Carruthers, RI, Jaronski, ST, Bradley, CA, Garza, CJ & Galaini-Wraight, S 2000,' Evaluation of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* for microbial control of the silverleaf whitefly, *Bemisia argentifolii*', *Biological Control*, vol. 17, no.3, pp. 203-217.

Xiao, Y, Avery, P, Chen, J, McKenzie, C & Osborne, L 2012,' Ornamental pepper as banker plants for establishment of *Amblyseius swirskii* (Acari: Phytoseiidae) for biological control of multiple pests in greenhouse vegetable production', *Biological Control*, vol. 63, no.3, pp. 279-286.

Yang, N-W & Wan, F-H 2011,' Host suitability of different instars of *Bemisia tabaci* biotype B for the parasitoid *Eretmocerus hyati*', *Biological Control*, vol.59, no.2, pp.313-317.

Yang, N-W., Li, A-L., Wan, F-H., Liu, W-X., Johnson, D. 2010,'Effects of plant essential oils on immature and adult sweetpotato whitefly, *Bemisia tabaci* biotype B'. *Crop Protection*. 29:1200-1207.

Yarahmadi, F, Rajabpour, A, Zandi Sohani, N & Ramezani, L 2013, 'Investigating contact toxicity of *Geranium* and *Artemisia* essential oils on *Bemisia tabaci* Gen', *Avicenna Journal of Phytomedicine*, vol. 3, no. 2, pp. 106-11.

Zaki, FN 2008, 'Field application of plant extracts against the aphid, *B. brassicae* and the whitefly, *B. tabaci* and their side effects on their predators and parasites', *Archives of Phytopathology and Plant Protection*, vol. 41, no. 6, pp. 462-6.

Zang, L-S & Liu, T-X 2008,'Host feeding of three parasitoid species on *Bemisia tabaci* biotype B and implications for whitefly biological control', *Entomolgia Experimentalis Et Applicata*, vol. 127, no,1, pp.55-63.

Zolnerowich, G & Rose, M 1998,' *Eretmocerus* Haldeman (Hymenoptera: Aphelinidae) imported and released in the United States for control of *Bemisia* (*tabaci* complex) (Homoptera: Aleyrodidae)', *Proceedings of the Entomological Society of Washington*, vol. 100, no. 2, pp. 310-23.

Appendices

Appendix1. Efficacy of Different Formulations against Different Stages of Silverleaf Whitefly

No.	Formulation	Concentration (%)	Mortality			Remarks
			Eggs (%)	Immatures (%)	Adults (%)	
1	Capryl glucoside (CG)	2	NE	H	L	Severe Phytotoxicity effect at 5% and 10%
		1	NE	H	L	
		0.5	NE	H	L	
		0.25	NE	H	L	
		0.125	NE	H	L	
		0.06	NE	L	L	
		0.03	NE	VL	L	
2	Decyl glucoside (DG)	2	NE	H	L	
		1	NE	H	L	
		0.5	NE	H	L	
		0.25	NE	H	L	
		0.125	NE	H	L	
		0.06	NE	M	M	
		0.03	NE	L	L	
3	Lauryl glucoside (LG)	2	NE	H	L	
		1	NE	H	L	
		0.5	NE	H	L	
		0.25	NE	H	L	
		0.125	NE	H	L	
		0.06	NE	M	L	
		0.03	NE	L	L	

H: High Mortality rates (75-100%), M: Moderate Mortality rates (50-75%), L: Low Mortality rates (20-50%), VL: Very Low Mortality rates (5-20%), NE: Not Effective (0-5%), _: Not tested.

No.	Formulation	Concentration (%)	Mortality			Remarks
			Eggs (%)	Immatures (%)	Adults (%)	
4	Lauryl sucroside (LS)	2	NE	H	L	
		1	NE	H	L	
		0.5	NE	H	L	
		0.25	NE	H	L	
		0.125	NE	H	L	
		0.06	NE	L	L	
		0.03	NE	VL	L	
5	Capryl glucoside and alpha- Tops (CG1)	2	NE	H	M	Severe Phytotoxicity effect at 5% and 10%
6	Capryl glucoside, alpha- Tops and 25% eugenol (CG2)	2	NE	H	M	
7	Capryl glucoside, alpha- Tops and 37.5% eugenol (CG3)	2	NE	H	M	
8	Capryl glucoside, alpha- Tops and 50% eugenol (CG4)	2	NE	H	M	
9	Lauryl glucoside and alpha- Tops (LG1)	2	NE	H	M	
10	Lauryl glucoside, alpha- Tops and 25% eugenol (LG2)	2	NE	H	M	
11	Lauryl glucoside, alpha- Tops and 37.5% eugenol (LG3)	2	NE	H	M	
12	Lauryl glucoside, alpha- Tops and 50% eugenol (LG4).	2	NE	H	M	
13	Diethylene glycol monomethyl ether, DEGME - Cellosolve acetate (100%)	2	NE	_	M	
		1.5	_	_	_	
		1	_	_	_	
		0.5	NE	_	L	
14	Diethylene glycon monomethyl ether, DEGME1 (100%)	2	NE	_	M	
		1.5	_	_	_	
		1	_	_	_	
		0.5	NE	_	L	

H: High Mortality rates (75-100%), M: Moderate Mortality rates (50-75%), L: Low Mortality rates (20-50%), VL: Very Low Mortality rates (5-20%), NE: Not Effective (0-5%), _: Not tested.

No.	Formulation	Concentration (%)	Mortality			Remarks
			Eggs (%)	Immatures (%)	Adults (%)	
15	Diethylene glycol monobutyl ether, DEGBE (100%)	2	NE	_	M	
		1.5	_	_	L	
		1	_	_	L	
		0.5	NE	_	M	
16	Diethylene glycol monobutyl ether acetate, DEGBEA (100%)	2	NE	_	M	
		1.5	_	_	M	
		1	_	_	M	
		0.5	NE	_	M	
17	Laureth – 7ethylene oxide-carboxylate as the sodium salt, LEOCS (30%)	2	NE	_	M	
		1.5	_	_	_	
		1	_	_	_	
		0.5	NE	_	NE	
18	Laureth – 7ethylene oxide-carboxylate as the triethanolamine salt, LEOCT (30%)	2	NE	_	M	
		1.5	_	_	M	
		1	_	_	L	
		0.5	NE	_	L	
19	Short chain polyglucoside, SCPG (50%)	2	L	_	M	
		1.5	_	_	M	
		1	_	_	M	
		0.5	NE	_	L	

H: High Mortality rates (75-100%), M: Moderate Mortality rates (50-75%), L: Low Mortality rates (20-50%), VL: Very Low Mortality rates (5-20%), NE: Not Effective (0-5%), _ : Not tested.

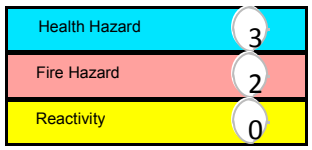
No.	Formulation	Concentration (%)	Mortality			Remarks
			Eggs (%)	Immatures (%)	Adults (%)	
20	Monoethanolamine (MEA)	2	NE	NE	H	Mild phytotoxicity effect at 0.25%
		1.5	NE	_	H	
		1	NE	_	H	
		0.5	NE	NE	H	
		0.25	_	_	H	
		0.1	_	_	L	
		0.05	_	_	L	
		0.025	_	_	VL	
21	Diethanolamine (DEA)	2	NE	NE	_	
		1.5	_	_	_	
		1	_	_	_	
		0.5	NE	NE	VL	
		0.25	_	_	_	
		0.1	_	_	_	
		0.05	_	_	_	
		0.025	_	_	_	
22	Triethanolamine(TEA)	2	NE	NE	_	Mild phytotoxicity effect at 0.25%
		1.5	_	_	_	
		1	_	_	_	
		0.5	NE	NE	VL	
		0.25	_	_	_	
		0.1	_	_	_	
		0.05	_	_	_	
		0.025	_	_	_	

H: High Mortality rates (75-100%), M: Moderate Mortality rates (50-75%), L: Low Mortality rates (20-50%), VL: Very Low Mortality rates (5-20%), NE: Not Effective (0-5%), _: Not tested.

No.	Formulation	Concentration (%)	Mortality			Remarks
			Eggs (%)	Immatures (%)	Adults (%)	
23	Monoisopropanolamine (MIPA)	2	NE	NE	H	
		1.5	_	_	H	
		1	_	_	H	
		0.5	NE	NE	H	
		0.25	_	_	H	
		0.1	_	_	M	
		0.05	_	_	L	
		0.025	_	_	VL	
24	Diisoprpanolamine (DIPA)	2	NE	NE	H	
		1.5	_	_	H	
		1	_	_	H	
		0.5	NE	NE	H	
		0.25	_	_	M	
		0.1	_	_	L	
		0.05	_	_	VL	
		0.025	_	_	VL	
25	Clove bud oil (90% eugenol)	0.01	NE	_	_	Moderate phytotoxicity effect at 0.01% and 0.025%
26	*Leptospermum petersonii*	0..005	NE	_	_	
27	*Lemon myrtle*	0.005	NE	_	_	
28	Gamma tops (gamma terpinene and alpha terpinene)	0.01	NE	_	_	
29	(50% mustard oil and 50% liquid soap)	0.25	L	_	L	Mild phytotoxicity effect at 1%
		0.5	M	_	M	
30	(75% mustard oil and 25% liquid soap)	0.25	H	_	L	
		1	H	_	H	

H: High Mortality rates (75-100%), M: Moderate Mortality rates (50-75%), L: Low Mortality rates (20-50%), VL: Very Low Mortality rates (5-20%), NE: Not Effective (0-5%), _: Not tested.

Appendix 2: Material Safety Data Sheet (MSDS) of mustard oil (Spectrum chemical 2006).

NFPA	HMIS	Personal Protective Equipment
		
		See Section 15.

Section 1. Chemical Product and Company Identification *Page Number: 1*

Common Name/ Trade Name	Mustard oil, natural
Manufacturer	SPECTRUM LABORATORY PRODUCTS INC. 14422 S. SAN PEDRO STREET GARDENA, CA 90248
Commercial Name(s)	Not available.
Synonym	Oil of Mustard; Oils, brassica alba; Oils, brassica nigra; Mustard Oils,
Chemical Name	Oils, Mustard
Chemical Family	Not available.
Chemical Formula	Not applicable.
Supplier	SPECTRUM LABORATORY PRODUCTS INC. 14422 S. SAN PEDRO STREET GARDENA, CA 90248

Catalog Number(s).	MU110
CAS#	8007-40-7
RTECS	RJ3694550
TSCA	TSCA 8(b) inventory: Mustard oil, natural; Allyl isothiocyanate
CI#	Not available.

IN CASE OF EMERGENCY
CHEMTREC (800) 424-9300

CALL (310) 516-8000

Section 2.Composition and Information on Ingredients

		Exposure Limits			
Name	CAS #	TWA (mg/m³)	STEL (mg/m³)	CEIL (mg/m³)	% by Weight
1) Allyl isothiocyanate, natural	57-06-7				93-98

Toxicological Data on Ingredients	Allyl Isothiocyanate, natural: ORAL (LD50): Acute: 112 mg/kg [Rat]. 308 mg/kg [Mouse]. DERMAL (LD50): Acute: 88 mg/kg [Rabbit].

Section 3. Hazards Identification

Potential Acute Health (irritant), Effects	Very hazardous in case of skin contact (permeator). Hazardous in case of skin contact (irritant), of eye contact of ingestion, of inhalation (lung irritant). Severe over-exposure can result in death.
Potential Chronic Health Effects	Slightly hazardous in case of skin contact (sensitizer), of inhalation. CARCINOGENIC EFFECTS: Classified 3 (Not classifiable for human.) by IARC [Allyl isothiocyanate, natural]. MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells. [Allyl isothiocyanate, natural]. Mutagenic for bacteria and/or yeast. [Allyl isothiocyanate, natural]. TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Not available. Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.

Section 4. First Aid Measures

Eye Contact	Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. WARM water MUST be used. Get medical attention.
Skin Contact	In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cover the irritated skin with an emollient. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.
Serious Skin Contact	Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.
Inhalation	If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.
Serious Inhalation	Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. WARNING: It may be hazardous to the person providing aid to give mouth-to-mouth resuscitation when the inhaled material is toxic, infectious or corrosive. Seek immediate medical attention.
Ingestion	If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.
Serious Ingestion	Not available.

Section 5. Fire and Explosion Data

Flammability of the Product	Flammable.
Auto-Ignition Temperature	Not available.
Flash Points	CLOSED CUP: 44.444°C (112°F).
Flammable Limits	Not available.
Products of Combustion	These products are carbon oxides (CO, CO2), nitrogen oxides (NO, NO2...).
Fire Hazards in Presence of Various Substances	Flammable in presence of open flames and sparks, of heat.

Explosion Hazards in Presence of Various Substances	Risks of explosion of the product in presence of mechanical impact: Not available. Risks of explosion of the product in presence of static discharge: Not available. Slightly explosive in presence of heat.
Fire Fighting Media and Instructions	Flammable liquid, soluble or dispersed in water. SMALL FIRE: Use DRY chemical powder. LARGE FIRE: Use alcohol foam, water spray or fog. Cool containing vessels with water jet in order to prevent pressure build-up, autoignition or explosion.
Special Remarks on Fire Hazards	Vapor may travel considerable distance to source of ignition and flash back. Contact with metals may evolve flammable hydrogen gas. Vapor may form explosive mixtures with air. When heated to decomposition it emits highly toxic fumes of cyanides.
Special Remarks on Explosion Hazards	Vapor may form explosive mixtures with air.

Section 6. Accidental Release Measures

Small Spill	Absorb with an inert material and put the spilled material in an appropriate waste disposal.

Section 7. Handling and Storage

Precautions	Keep away from heat. Keep away from sources of ignition. Ground all equipment containing material. Do not ingest. Do not breathe gas/fumes/ vapor/spray. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, acids, alkalis.
Storage	Store in a segregated and approved area. Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition (spark or flame). Sensitive to light. Store in light-resistant containers.

Section 8. Exposure Controls/Personal Protection

Engineering Controls	Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.
Personal Protection	Splash goggles. Lab coat. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.
Personal Protection in Case of a Large Spill	Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.
Exposure Limits	Allyl isothiocyanate, natural STEL: 1 from AIHA [United States] Inhalation STEL: 4 (mg/m^3) from AIHA [United States] Inhalation Consult local authorities for acceptable exposure limits.

Section 9. Physical and Chemical Properties

Physical state and appearance	Liquid. (Oily liquid.)	Odor	Pungent mustard. Irritant.
		Taste	Sharp biting.
Molecular Weight	Not applicable.	Color	Colorless to light yellow.

pH (1% soln/water)	Not available.
Boiling Point	148°C (298.4°F) - 154 C.
Melting Point	-80°C (-112°F)
Critical Temperature	Not available.
Specific Gravity	1.008 - 1.02(Water = 1)
Vapor Pressure	0.5 kPa (@ 20°C)
Vapor Density	3.41 (Air = 1)
Volatility	Not available.
Odor Threshold	The highest known value is 0.008 ppm (Allyl isothiocyanate)
Water/Oil Dist. Coeff.	Not available.
Ionicity (in Water)	Not available.
Dispersion Properties	See solubility in water, diethyl ether.
Solubility	Soluble in diethyl ether. Slightly soluble in cold water. Solubility in Water: 5% @ 20 deg. C. Completely miscible with chloroform, benzene. Miscible with alcohol and most organic solvents. Solubility in 70% alcohol: 1 ml dissolves in 10 ml. Solubility in 80% Ethanol: 1 ml dissolves in 8 ml. Soluble in Carbon disulfide.

Section 10. Stability and Reactivity Data

Stability	The product is stable.
Instability Temperature	Not available.
Conditions of Instability	Heat, ignition sources (flames, sparks, etc.), incompatible materials
Incompatibility with various substances	Reactive with oxidizing agents, acids, alkalis.
Corrosivity	Non-corrosive in presence of glass.
Special Remarks on Reactivity	Tends to darken on aging. Incompatible with amines, alcohols. Dangerous on contact with acids or acid fumes. It emits highly toxic fumes of cyanides. Sensitive to light.
Special Remarks on Corrosivity	Not available.
Polymerization	Will not occur.

Section 11. Toxicological Information

Routes of Entry	Absorbed through skin. Dermal contact. Eye contact. Inhalation. Ingestion.

Toxicity to Animals	Acute oral toxicity (LD50): 117 mg/kg (Rat) (Calculated value for the mixture). Acute dermal toxicity (LD50): 92 mg/kg (Rabbit) (Calculated value for the mixture).
Chronic Effects on Humans	CARCINOGENIC EFFECTS: Classified 3 (Not classifiable for human.) by IARC [Allyl isothiocyante, natural]. MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells. [Allyl isothiocyanate, natural]. Mutagenic for bacteria and/or yeast. [Allyl isothiocyanate, natural].
Other Toxic Effects on Humans	Very hazardous in case of skin contact (permeator). Hazardous in case of skin contact (irritant), of ingestion, of inhalation (lung irritant).
Special Remarks on Toxicity to Animals	Not available.
Special Remarks on Chronic Effects on Humans	May affect genetic material (mutagenic). May cause adverse reproductive effects. May cause cancer based on animal test data (Allyl isothiocyanate, natural)
Special Remarks on other Toxic Effects on Humans	Acute Potential Health Effects: Skin: Causes skin irritation and possible blistering and burns. May be fatal if absorbed through skin. Eyes: Causes eye irritation. Lachrymator (substance which increases the flow of tears). Inhalation: Causes respiratory tract irritation. Ingestion: Harmful if swallowed. Causes gastroenteritis (gastrointestinal tract irritation) with nausea vomiting, diarrhea. Chronic Potential Health Effects: Skin: Prolonged or repeated skin contact may cause skin sensitization, an allergic reaction.\ Inhalation: Prolonged or repeated inhalation of vapor or mist may cause allergy type reaction to develop with watery eyes, sneezing, runny nose and symptoms of asthma (coughing, wheezing, and chest tightness). One allery develops, even very limited exposures can cause symptoms to develop. Ingestion: Prolonged or repeated ingestion of larger amounts may affect the liver, blood (changes in serum composition), and behavior/central nervous system (somnolence).

Section 12. Ecological Information

Ecotoxicity	Not available.
BOD5 and COD	Not available.
Products of Biodegradation	Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.
Toxicity of the Products of Biodegradation	The products of degradation are less toxic than the product itself.
Special Remarks on the Products of Biodegradation	Not available.

Section 13. Disposal Considerations

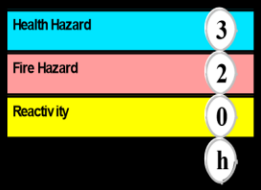
Waste Disposal	Waste must be disposed of in accordance with federal, state and local environmental control regulations.

Section 14. Transport Information

DOT Classification	CLASS 3: Flammable liquid. CLASS 6.1: Poisonous material.
Identification	: Allyl isothiocyanate, stabilized (Mustard oil, synthetic) UNNA: 1545 PG: II
Special Provisions for Transport	Not available.

DOT (Pictograms)

HMIS (U.S.A.)



National Fire Protection Association (U.S.A.)

WHMIS (Canada) (Pictograms)

DSCL (Europe) (Pictograms)

TDG (Canada) (Pictograms)

ADR (Europe) (Pictograms)

Protective Equipment

Appendix 3: Material Safety Data Sheet (MSDS) of Trix® (Micon national 2005).

Section 1 - CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

PRODUCT NAME

TRIX DISHWASHING LIQUID - LEMON

SYNONYMS

domestic dishwashing liquid

PRODUCT USE

Manual dishwashing liquid.

SUPPLIER

Company: United Laboratories Address:

282 Hammond Rd

Dandenong

VIC, 3175

AUSTRALIA

Telephone: (+61 3) 9794 3333

Emergency Tel: 1800 809 282 Fax: 03 9794 3301

HAZARD RATINGS

SCALE: Min/Nil=0 Low=1 Moderate=2 High=3 Extreme=4

Section 2 - HAZARDS IDENTIFICATION

STATEMENT OF HAZARDOUS NATURE

NON-HAZARDOUS SUBSTANCE. NON-DANGEROUS GOODS.

According to the Criteria of NOHSC, and the ADG Code.

POISONS SCHEDULE

None

RISK

SAFETY

Do not breathe gas/fumes/vapour/spray.
Avoid contact with skin.

Section 3 - COMPOSITION / INFORMATION ON INGREDIENTS

NAME	CAS RN	%
sodium dodecylbenzenesulfonate	25155-30-0	<10
sodium lauryl ether sulfate	9004-82-4	<10
Isothiazolinone		<0.2
non-hazardous ingredients		balance

Section 4 - FIRST AID MEASURES

SWALLOWED

- Immediately give a glass of water.
- First aid is not generally required. If in doubt, contact a PoisonsInformation Centre or a doctor.

EYE

If this product comes in contact with eyes:

- Wash out immediately with water.
- If irritation continues, seek medical attention.
- Removal of contact lenses after an eye injury should only be undertaken byskilled personnel.

SKIN

If skin contact occurs:

- Immediately remove all contaminated clothing, including footwear
- Flush skin and hair with running water (and soap if available).
- Seek medical attention in event of irritation.

INHALED

- If fumes or combustion products are inhaled remove from contaminated area.
- Other measures are usually unnecessary.

NOTES TO PHYSICIAN

Treat symptomatically.

Section 5 - FIRE FIGHTING MEASURES

EXTINGUISHING MEDIA

• There is no restriction on the type of extinguisher which may be used.

Use extinguishing media suitable for surrounding area

FIRE FIGHTING

• Alert Fire Brigade and tell them location and nature of hazard.
• Wear breathing apparatus plus protective gloves for fire only.
• Prevent, by any means available, spillage from entering drains or watercourses.
• Use fire fighting procedures suitable for surrounding area.
• DO NOT approach containers suspected to be hot.
• Cool fire exposed containers with water spray from a protected location.
• If safe to do so, remove containers from path of fire.
• Equipment should be thoroughly decontaminated after use.

FIRE/EXPLOSION HAZARD

• Non combustible.
• Not considered to be a significant fire risk.
• Expansion or decomposition on heating may lead to violent rupture ofcontainers.
• Decomposes on heating and may produce toxic fumes of carbon monoxide (CO).
• May emit acrid smoke.

Decomposition may produce toxic fumes of. carbon dioxide (CO_2).
sulfur oxides (SOx). other pyrolysis products typical of burning organic material

FIRE INCOMPATIBILITY

None known

HAZCHEM

None

Personal Protective Equipment

PERSONAL PROTECTION EQUIPMENT

Breathing apparatus.

Gas tight chemical resistant suit.

Section 6 - ACCIDENTAL RELEASE MEASURES

EMERGENCY PROCEDURES

MINOR SPILLS

Slippery when spilt.

- Clean up all spills immediately.
- Avoid breathing vapours and contact with skin and eyes.
- Control personal contact by using protective equipment.
- Contain and absorb spill with sand, earth, inert material or vermiculite.
- Wipe up.
- Place in a suitable labelled container for waste disposal.

MAJOR SPILLS

Slippery when spilt. Minor hazard.

- Clear area of personnel.

- Alert Fire Brigade and tell them location and nature of hazard.
- Control personal contact by using protective equipment as required.
- Prevent spillage from entering drains or water ways.
- Contain spill with sand, earth or vermiculite.
- Collect recoverable product into labelled containers for recycling.
- Absorb remaining product with sand, earth or vermiculite and place inappropriate containers for disposal.
- Wash area and prevent runoff into drains or waterways.
- If contamination of drains or waterways occurs, advise emergency services.

Personal Protective Equipment advice is contained in Section 8 of the MSDS.

Section 7 - HANDLING AND STORAGE

PROCEDURE FOR HANDLING

- Limit all unnecessary personal contact.
- Wear protective clothing when risk of exposure occurs.
- Use in a well-ventilated area.
- When handling DO NOT eat, drink or smoke.
- Always wash hands with soap and water after handling.
- Avoid physical damage to containers.
- Use good occupational work practice.
- Observe manufacturer's storing and handling recommendations.

DO NOT allow clothing wet with material to stay in contact with skin

SUITABLE CONTAINER

- Polyethylene or polypropylene container.
- Packing as recommended by manufacturer
- Check all containers are clearly labelled and free from leaks.

STORAGE INCOMPATIBILITY

Avoid reaction with oxidising agents

STORAGE REQUIREMENTS

- Store in original containers.
- Keep containers securely sealed.
- Store in a cool, dry, well-ventilated area.
- Store away from incompatible materials and foodstuff containers.
- Protect containers against physical damage and check regularly for leaks.
- Observe manufacturer's storing and handling recommendations.

Section 8 - EXPOSURE CONTROLS / PERSONAL PROTECTION

EXPOSURE CONTROLS

No data available for sodium dodecylbenzenesulfonateas (CAS: 25155-30-0) / (CAS: 9004-82-4)

ODOUR SAFETY FACTOR (OSF)

OSF=0.36 (FORMALDEHYDE)

Exposed individuals are NOT reasonably expected to be warned, by smell, that the Exposure Standard is being exceeded.

Odour Safety Factor (OSF) is determined to fall into either Class C, D or E.
The Odour Safety Factor (OSF) is defined as:

OSF= Exposure Standard (TWA) ppm/ Odour Threshold Value (OTV) ppm

Classification into classes follows:

Class	OSF	Description
A	550	Over 90% of exposed individuals are aware by smell that the Exposure Standard (TLV-TWA for example) is being reached, ev when distracted by working activities
B	26-550	As "A" for 50-90% of persons being distracted

C	1-26	As "A" for less than 50% of persons being distracted
D	0.18-1	10-50% of persons aware of being tested perceive by smell tha Exposure Standard is being reached
E	<0.18	As "D" for less than 10% of persons aware of being tested

INGREDIENT DATA

SODIUM DODECYLBENZENESULFONATE:

TLV TWA: 10 mg/m³ (Value for particulate matter containing no asbestos and <1% crystalline silica,Inhalable fraction) [ACGIH]

TLV TWA: 3 mg/m³ (Value for particulate matter containing no asbestos and <1% crystalline silica,Respirable fraction) [ACGIH]

Dusts not otherwise classified, as inspirable
dust; ES TWA: 10 mg/m³

SODIUM LAURYL ETHER SULFATE:

No exposure limits set by NOHSC or ACGIH

PERSONAL PROTECTION

EYE

No special equipment for minor exposure i.e. when handling small quantities.

- OTHERWISE:
- Safety glasses with side shields.
- Contact lenses pose a special hazard; soft lenses may absorb irritants and all lenses concentrate them.

HANDS/FEET

No special equipment needed when handling small quantities.
OTHERWISE: Wear chemical protective gloves, eg. PVC.

OTHER

No special equipment needed when handling small quantities.

OTHERWISE:

- Overalls.

- Barrier cream.
- Eyewash unit.

ENGINEERING CONTROLS

General exhaust is adequate under normal operating conditions. If risk of overexposure exists, wear SAA approved respirator. Correct fit is essential to obtain adequate protection. Provide adequate ventilation in warehouse or closed storage areas. Air contaminants generated in the workplace possess varying "escape" velocities which, in turn, determine the "capture velocities" of fresh circulating air required to effectively remove the contaminant.

Type of Contaminant:	Air Speed:
solvent, vapours, degreasing etc., evaporating from tank (in still air)	0.25-0.5 m/s (50-100 f/min)
aerosols, fumes from pouring operations, intermittent container filling, low speed conveyer transfers, welding, spray drift, plating acid fumes, pickling (released at low velocity into zone of active generation)	0.5-1 m/s (100-200 f/min.)
direct spray, spray painting in shallow booths, drum filling, conveyer loading, crusher dusts, gas discharge (active generation into zone of rapid air motion)	1-2.5 m/s (200-500 f/min)
grinding, abrasive blasting, tumbling, high speed wheel generated dusts (released at high initial velocity into zone of very high rapid air motion).	2.5-10 m/s (500-2000 f/min.)

Within each range the appropriate value depends on:

Lower end of the range	Upper end of the range
1: Room air currents minimal or favourable to capture	1: Disturbing room air currents
2: Contaminants of low toxicity or of nuisance value only	2: Contaminants of high toxicity
3: Intermittent, low production.	3: High production, heavy use
4: Large hood or large air mass in motion	4: Small hood - local control only

Simple theory shows that air velocity falls rapidly with distance away from the opening of a simple extraction pipe. Velocity generally decreases with the square of distance from the extraction point (in simple cases). Therefore the air speed at the extraction point should be adjusted, accordingly, after reference to distance from the contaminating source. The air velocity at the extraction fan, for example, should be a minimum of 1-2 m/s (200-400 f/min.) for extraction of solvents generated in a tank 2 meters distant from the extraction point. Other mechanical considerations, producing performance deficits within the extraction apparatus, make it

essential that theoretical air velocities are multiplied by factors of 10 or more when extraction systems are installed or used.

Section 9 - PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE

Transparent yellow liquid; mixes with water.

PHYSICAL PROPERTIES

Liquid.

Mixes with water.

Molecular Weight: Not Applicable Boiling Range (°C): 100 (water)

Melting Range (°C): Not Available Specific Gravity (water=1): 1.03 Solubility in water (g/L): Miscible pH (as supplied): 8.5

pH (1% solution): Not Available Vapour Pressure (kPa): 2.3 @ 20 deg C

Volatile Component (%vol): Not Available Evaporation Rate: Not Available

Relative Vapour Density (air=1): Not Available Flash Point (°C): >65

Lower Explosive Limit (%): Not Applicable Upper Explosive Limit (%): Not Applicable
Autoignition Temp (°C): Not Applicable Decomposition Temp (°C): Not Available

State: Liquid

Section 10 - CHEMICAL STABILITY AND REACTIVITY INFORMATION

CONDITIONS CONTRIBUTING TO INSTABILITY

- Presence of incompatible materials.
- Product is considered stable.
- Hazardous polymerisation will not occur.

Section 11 - TOXICOLOGICAL INFORMATION

POTENTIAL HEALTH EFFECTS

ACUTE HEALTH EFFECTS SWALLOWED

The material has NOT been classified by EC Directives or other classification systems as "harmful by ingestion". This is because of the lack of corroboratin animal or human evidence. The material may still be damaging to the health of the individual, following ingestion, especially where pre-existing organ (e.g liver, kidney) damage is evident. Present definitions of harmful or toxic substances are generally based on doses producing mortality rather than those producing morbidity (disease, ill-health). Gastrointestinal tract discomfort may produce nausea and vomiting. In an occupational setting however, ingestion of insignificant quantities is not thought to be cause for concern.

EYE

Although the liquid is not thought to be an irritant (as classified by EC Directives), direct contact with the eye may produce transient discomfort characterised by tearing or conjunctival redness (as with windburn).

SKIN

Entry into the blood-stream, through, for example, cuts, abrasions or lesions, may produce systemic injury with harmful effects. Examine the skin prior to the use of the material and ensure that any external damage is suitably protected. The material is not thought to produce adverse health effects or skin irritation following contact (as classified by EC Directives using animal models).

Nevertheless, good hygiene practice requires that exposure be kept to a minimum and that suitable gloves be used in an occupational setting.

INHALED

The material is not thought to produce adverse health effects or irritation of the respiratory tract (as classified by EC Directives using animal models). Nevertheless, good hygiene practice requires that exposure be kept to a minimum and that suitable control measures be used in an occupational setting.

CHRONIC HEALTH EFFECTS

Limited evidence shows that inhalation of the material is capable of inducing a sensitisation reaction in a significant number of individuals at a greater frequency than would be expected from the response of a normal population. Pulmonary sensitisation, resulting in hyperactive airway dysfunction and pulmonary allergy may be accompanied by fatigue, malaise and aching. Significant symptoms of exposure may persist for extended periods, even after exposure ceases. Symptoms can be activated by a variety of nonspecific environmental stimuli such as automobile exhaust, perfumes and passive smoking. . There exists limited evidence that shows that skin contact with the material is capable either of inducing a sensitisation reaction in a

significant number of individuals, and/or of producing positive response in experimental animals. Absorbed sulfonates are quickly distributed through living systems and are readily excreted. Toxic effects may result from the effects of binding to proteins and the ability of sulfonates to translocate potassium and nitrate (NO3-) ions from cellular to interstitial fluids. Airborne sulfonates may be responsible for respiratory allergies and, in some instances, minor dermal allergies.

Trix Dishwashing Liquid - Lemon

Not available. Refer to individual constituents.

unless otherwise specified data extracted from RTECS - Register of Toxic Effects of Chemical Substances

SODIUM DODECYLBENZENESULFONATE:

TOXICITY	IRRITATION
Oral (rat) LD50: 438 mg/kg	Skin (rabbit): 20 mg/24 hr-SEVERE
	Eye (rabbit): 0.25 mg/24hr-SEVERE
	Eye (rabbit): 1% - SEVERE

SODIUM LAURYL ETHER SULFATE:

TOXICITY	IRRITATION
Oral (rat) LD50: 1600 mg/kg	Skin (rabbit):25 mg/24 hr moderate

Section 12 - ECOLOGICAL INFORMATION

Octanol/water partition coefficients cannot easily be determined for surfactants because one part of the molecule is hydrophilic and the other part is hydrophobic. Consequently they tend to accumulate at the interface and are not extracted into one or other of the liquid phases. As a result surfactants are expected to transfer slowly, for example, from water into the flesh of fish. During this process, readily biodegradable surfactants are expected to be metabolised rapidly during the process of bioaccumulation. This was emphasised by the OECD Expert Group stating that chemicals are not to be considered to show bioaccumulation potential if they are readily biodegradable.

Several anionic and nonionic surfactants have been investigated to evaluate their potential to bioconcentrate in fish. BCF values (BCF - bioconcentration factor) ranging from 1 to 350 were found. These are absolute maximum values, resulting from the radiolabelling technique used. In all these studies, substantial oxidative metabolism was found resulting in the highest radioactivity in the gall bladder. This indicates liver transformation of the parent compound and biliary excretion

of the metabolised compounds, so that "real" bioconcentration is overstated. After correction it can be expected that "real" parent BCF values are one order of magnitude less than those indicated above, i.e. "real" BCF is <100. Therefore the usual data used for classification by EU directives to determine whether a substance is "Dangerous to the "Environment" has little bearing on whether the use of the surfactant is environmentally acceptable.

DO NOT discharge into sewer or waterways.

Section 13 - DISPOSAL CONSIDERATIONS

- Recycle wherever possible or consult manufacturer for recycling options.
- Consult State Land Waste Management Authority for disposal.
- Bury residue in an authorised landfill.
- Recycle containers if possible, or dispose of in an authorised landfill.

Section 14 - TRANSPORTATION INFORMATION

Shipping Name:

None

Dangerous Goods Class: None

UN/NA Number: None

ADR Number: None

Section 14 - TRANSPORTATION INFORMATION ...

Packing Group: None Additional
Shipping Information:

International Transport Regulations:

IMO: None

HAZCHEM

None

Section 15 - REGULATORY INFORMATION

POISONS SCHEDULE

None